

Fig. 1

2H7scFv-Ig cDNA and predicted amino acid sequence:

HindIII NcoI 2H7 V_L Leader Peptide→
~~~~~                      ~~~~~

1 AAGCTTGGCCG CCATGGATT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA  
M D F Q V Q I F S F L L I S A S

61 GTCATAATTG CCAGAGGACA AATTGTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT  
V I I A R G Q I V L S Q S P A I L S A S

121 CCAGGGGAGA AGGTCACAA TACTGTCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG  
P G E K V T M T C R A S S S V S Y M H W

BamHI  
~~~~~

181 TACCAGCAGA AGCCAGGATC CTCCCCCAA CCCTGGATTT ATGCCCATC CAACCTGGCT
Y Q Q K P G S S P K P W I Y A P S N L A

241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAAATC
S G V P A R F S G S G S G T S Y S L T I

301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA
S R V E A E D A A T Y Y C Q Q W S F N P

(Gly₄Ser)₃ Linker

361 CCCACGTTCC GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT
P T F G A G T K L E L K G G G G S G G G

421 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG
G S G G G G S S Q A Y L Q Q S G A E L V

481 AGGCCTGGGG CCTCAGTGAA GATGTCTCTG AAGGCTTCTG GCTACACATT TACCAGTTAC
R P G A S V K M S C K A S G Y T F T S Y

541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT
N M H W V K Q T P R Q G L E W I G A I Y

601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA
P G N G D T S Y N Q K F K G K A T L T V

661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG
D K S S S T A Y M Q L S S L T S E D S A

721 GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG
V Y F C A R V V Y Y S N S Y W Y F D V W

Fig. 1 (continued)

BclI
 ~~~~~human IgG1 Fc domain →

781     G T G T T V T V S D Q E P K S C D K T H  
 GGCACAGGGA CCACGGTCAC CGTCTCTGAT CAGGAGCCCA AATCTTGTGA CAAAACCTCAC

841     T C P P C P A P E L L G G P S V F L F P  
 ACATGCCCAC CGTGCCCGAGC ACCTGAACTC CTGGGGGGGAC CGTCAGTCTT CCTCTTCCCC

901     P K P K D T L M I S R T P E V T C V V V  
 CCAAACCCA AGGACACCCT CATGATCTCC CGGACCCCTG AGGTCACATG CGTGGTGGTG

961     D V S H E D P E V K F N W Y V D G V E V  
 GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG

1021    H N A K T K P R E E Q Y N S T Y R V V S  
 CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC

1081    V L T V L H Q D W L N G K E Y K C K V S  
 GTCCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC

1141    N K A L P A P I E K T I S K A K G Q P R  
 AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACAATCTCCA AAGCCAAAGG GCAGCCCCGA

1201    E P Q V Y T L P P S R D E L T K N Q V S  
 GAACCACAGG TGTACACCCT GCCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTCAGC

1261    L T C L V K G F Y P S D I A V E W E S N  
 CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT

1321    G Q P E N N Y K T T P P V L D S D G S F  
 GGGCAGCCGG AGAACAATA CAAGACCACG CCTCCCGTGC TGGACTCCGA CGGCTCCTTC

1381    F L Y S K L T V D K S R W Q Q G N V F S  
 TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA

1441    C S V M H E A L H N H Y T Q K S L S L S  
 TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT

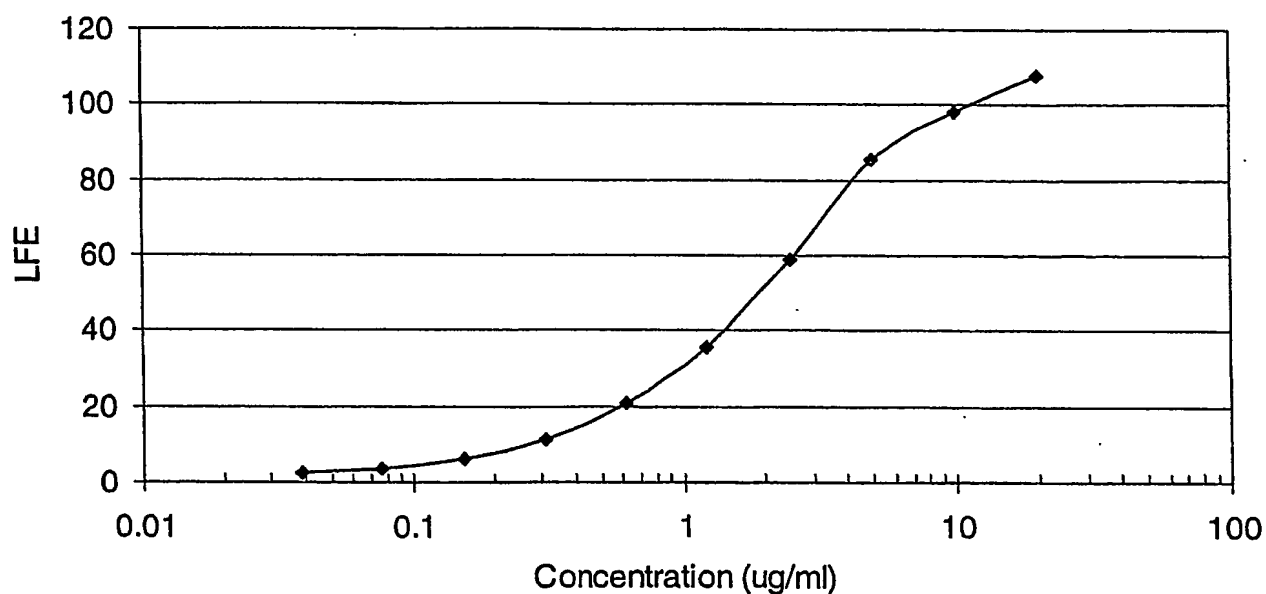
XbaI  
 ~~~~~

1501 P G K * S R
 CCGGGTAAAT GATCTAGA

Fig. 2

Production Levels of 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3
by Stable CHO Lines

2H7scFvlg Standard Curve



Clone	LFE @ 1:50 Estimated Concentration (µg/ml)
D2	26.156
IIIC6	25.755
IVA3	28.661
Spent bulk	29.664

Fig. 3

SDS-PAGE Analysis of
2H7 scFvIgG1 (SSS-S)H WCH2 WCH3 Protein.

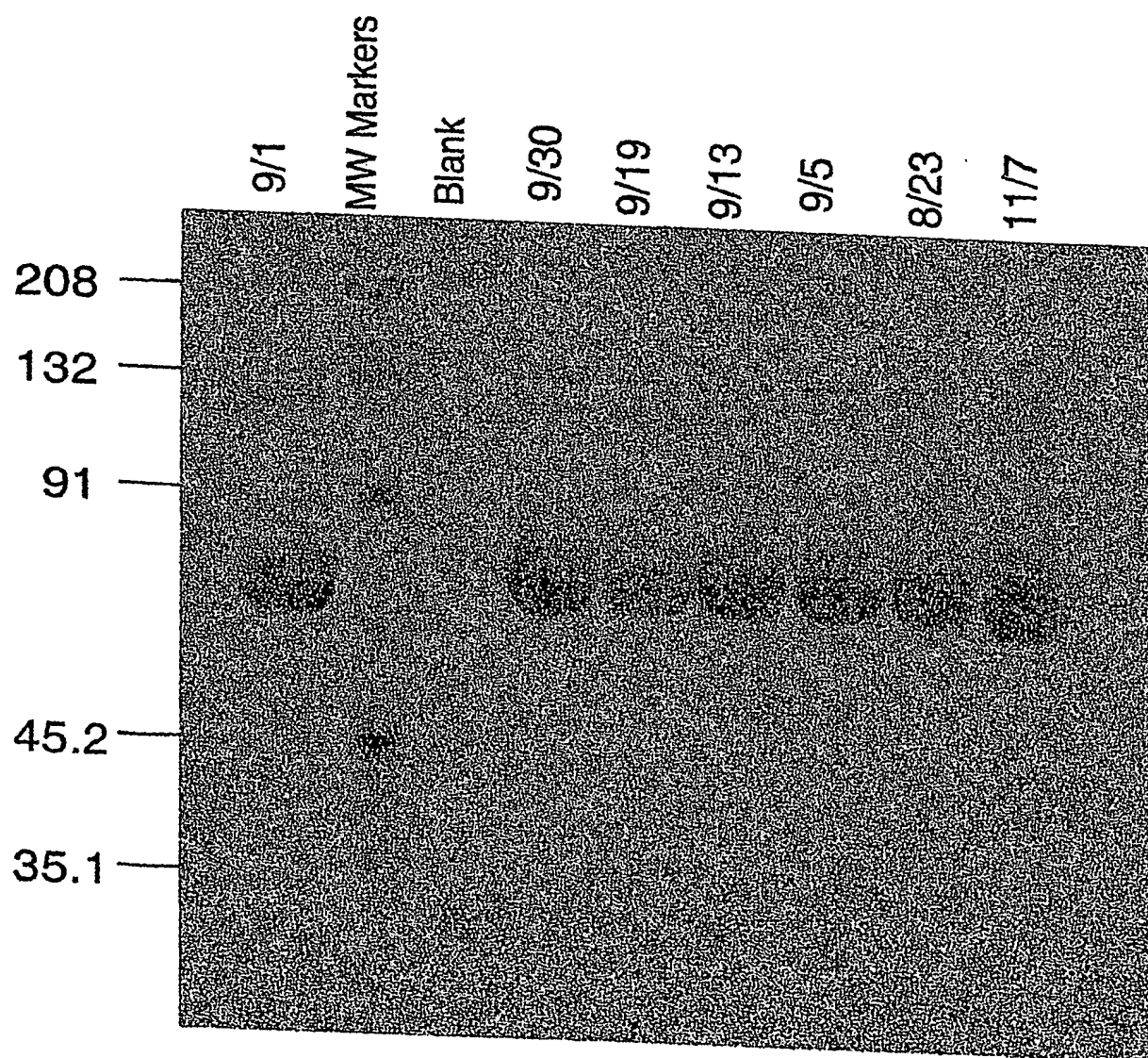


Fig. 4A

Complement Mediated B Cell Killing After Binding of
CD20-targeted 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3:

2H7scFv-Ig Concentration	RAMOS # live cells/total cells		BJAB # live cells/total cells	
20 µg/ml + complement	-	0.16	-	0.07
5 µg/ml + complement	-	0.2	-	N.D.
1.25 µg/ml + complement	-	0.32	-	0.1
Complement alone	-	0.98	-	0.94

*Viability was determined by trypan blue exclusion and is tabulated as the fraction of viable cells out of the total number of cells counted.

**N.D. (not determined).

Fig. 4B

Antibody-dependent cellular cytotoxicity (ADCC) mediated by 2H7scFv-IgG1 (SSS-S)H WCH2 WCH3:

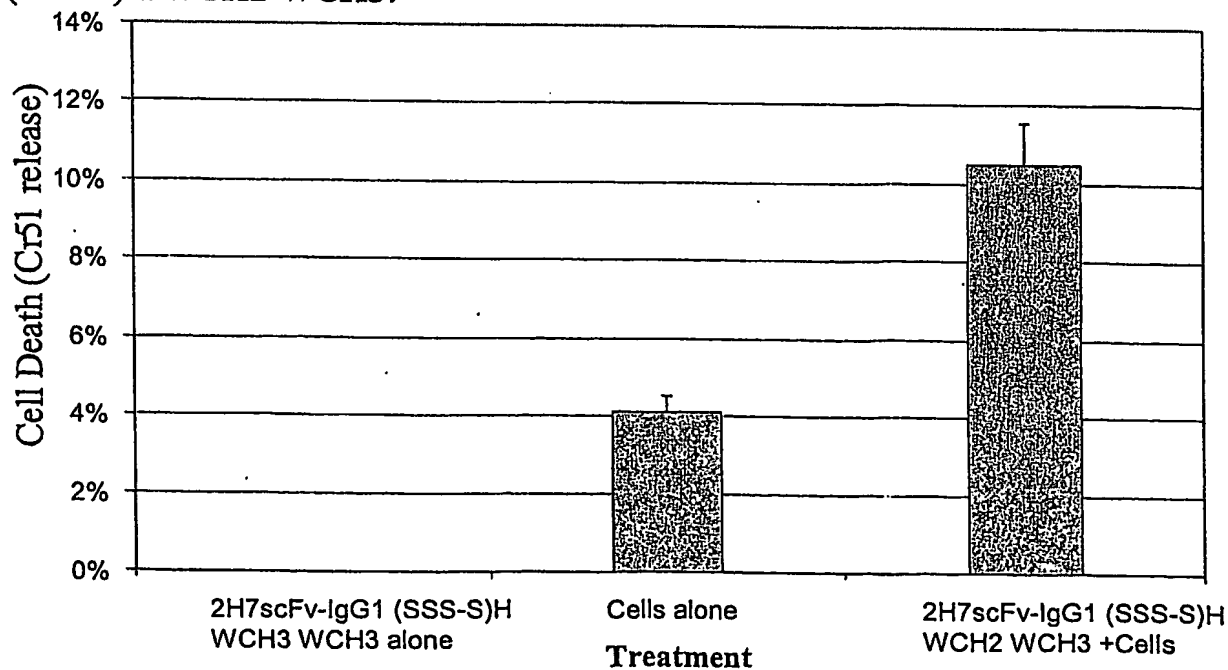


Fig. 5

Effects of Crosslinking of CD20 and CD40 Cell Surface Receptors
on B Cell Proliferation:

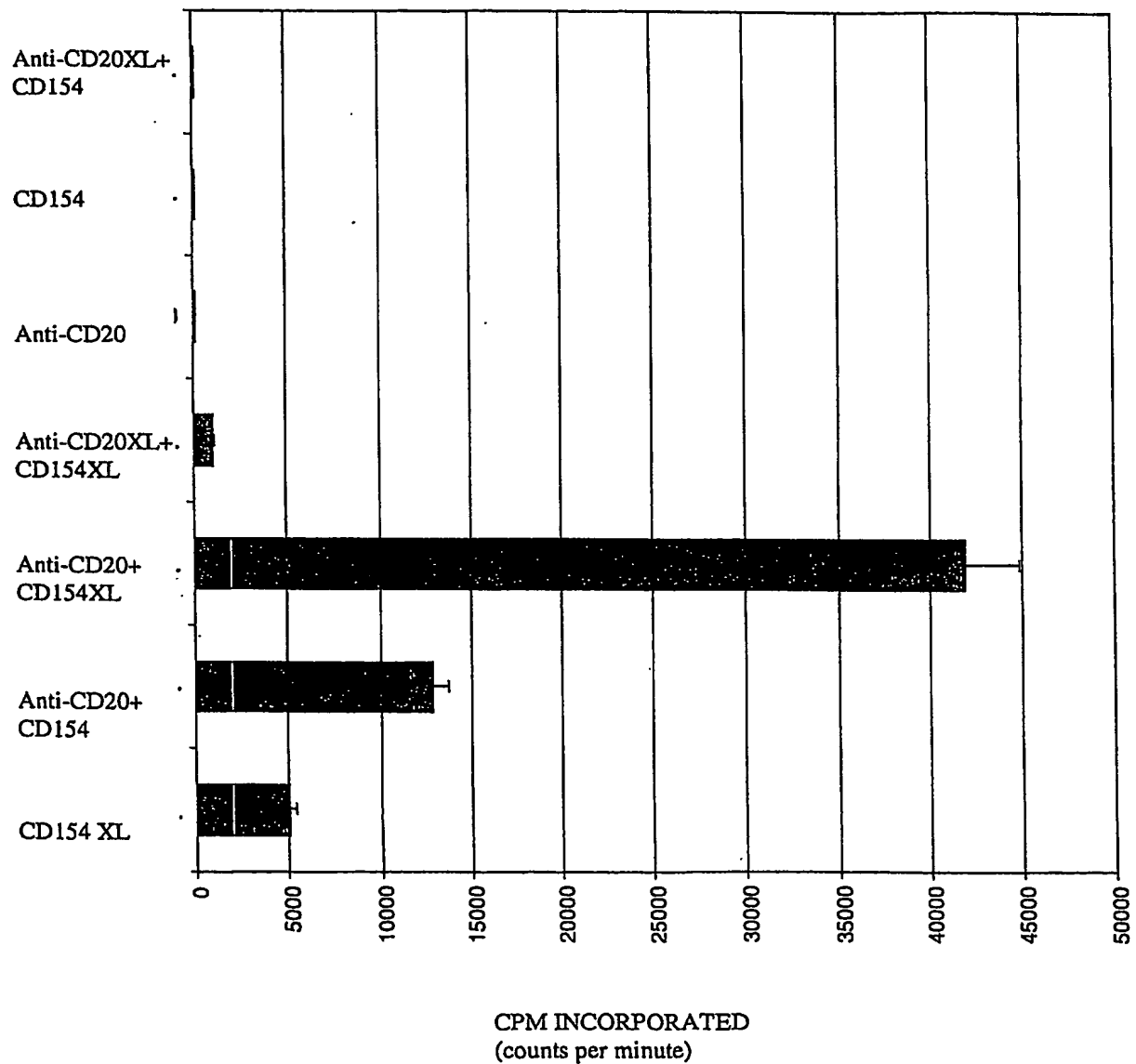


Fig. 6

Effect of Simultaneous ligation of CD20 and CD40
on CD95 and apoptosis.

Fig. 6A.

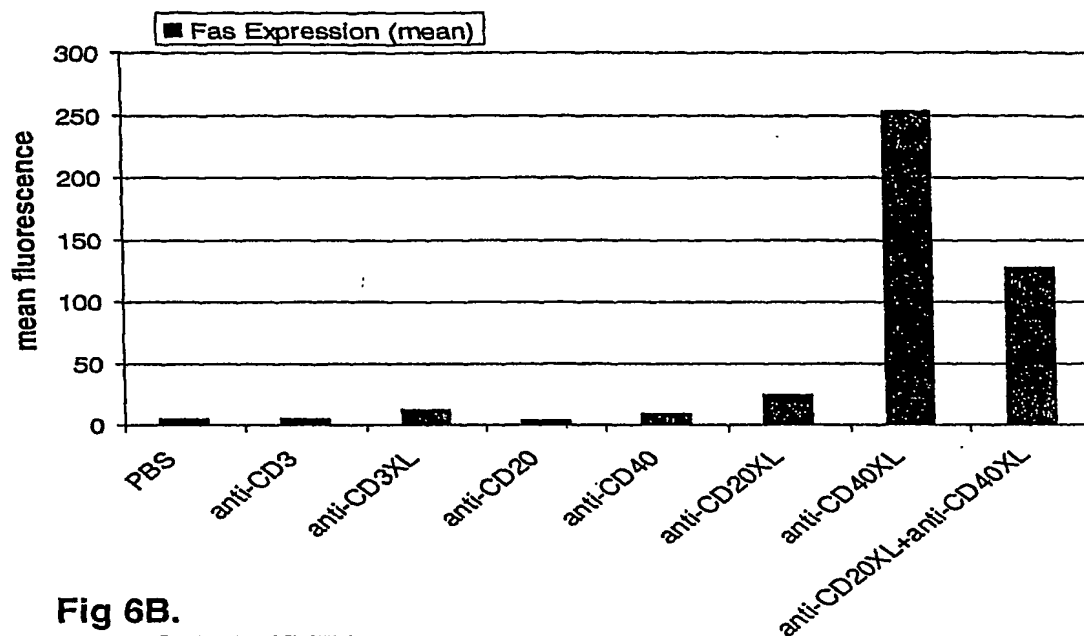
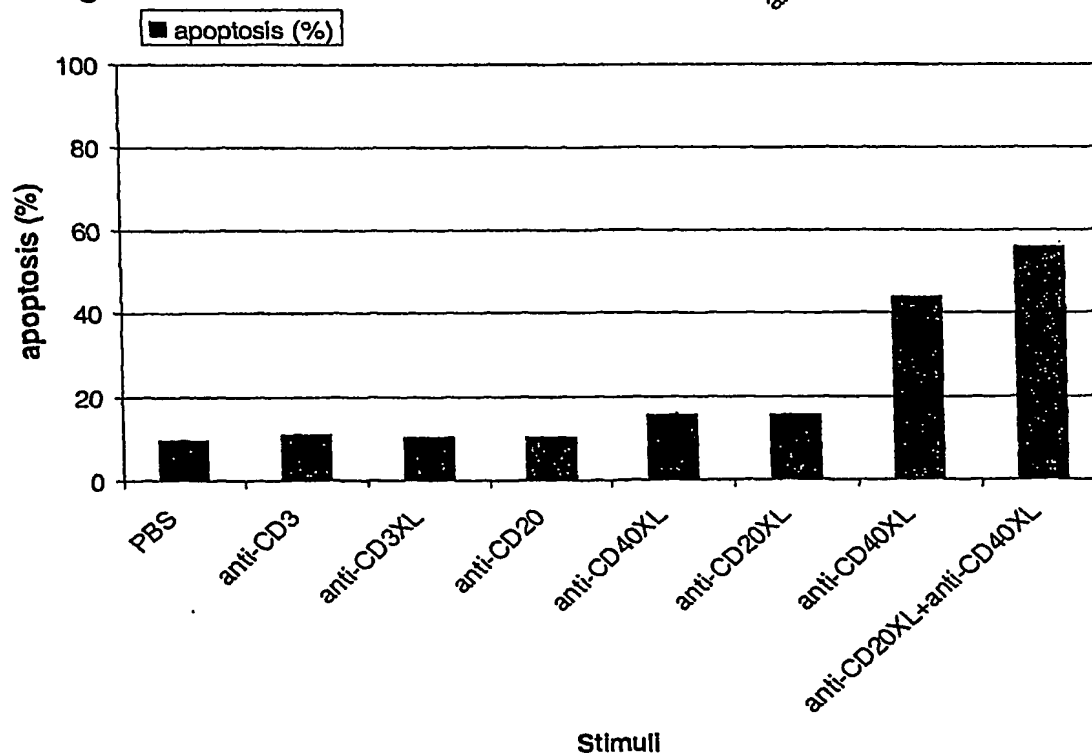


Fig 6B.



2H7-CD154 L2 cDNA and predicted amino acid sequence:

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Fig. 7A (continued)

human CD154/amino acid 48→

Bcl/Bam hybrid site

781 G T G T T V T V S D P R R L D K I E D E
GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAAGAAGGT TGGACAAGAT AGAAGATGAA

841 R N L H E D F V F M K T I Q R C N T G E
AGGAATCTTC ATGAAGATTT TGTATTCATG AAAACGATAC AGAGATGCAA CACAGGAGAA

901 R S L S L L N C E E I K S Q F E G F V K
AGATCCTTAT CCTTACTGAA CTGTGAGGAG ATTAAGAGCC AGTTTGAAGG CTTTGTGAAG

BclI

961 D I M L N K E E T K K E N S F E M Q K G
GATATAATGT TAAACAAAGA GGAGACGAAG AAAGAAAACA GCTTTGAAAT GCAAAAAGGT

BclI
~~~~~

1021 D Q N P Q I A A H V I S E A S S K T T S  
GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAAACATCT

1081 V L Q W A E K G Y Y T M S N N L V T L E  
GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCTTGGA

1141 N G K Q L T V K R Q G L Y Y I Y A Q V T  
AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

HindIII  
~~~~~

1201 F C S N R E A S S Q A P F I A S L C L K
TTCTGTTCCA ATCGGGAAGC TTCGAGTCAA GCTCCATTTA TAGCCAGCCT CTGCCTAAAG

1261 S P G R F E R I L L R A A N T H S S A K
TCCCCGGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

1321 P C G Q Q S I H L G G V F E L Q P G A S
CCTTGCGGGC AACAAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

NcoI
~~~~~

1381 V F V N V T D P S Q V S H G T G F T S F  
GTGTTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCCTT

XhoI XbaI  
~~~~~

1441 G L L K L E * * S R
GGCTTACTCA AACTCGAGTG ATAATCTAGA

Fig. 7B.

2H7scFv-CD154 S4 cDNA and predicted amino acid sequence:

HindIII NcoI
 ~~~~~ ~~~~~2H7 V<sub>L</sub> Leader Peptide→  
 M D F Q V Q I F S F L L I S A S  
 1 AAGCTTGCCG CC ATGGATTT TCAAGTCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

2H7 V<sub>L</sub> →  
 V I I A R G Q I V L S Q S P A I L S A S  
 61 GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

P G E K V T M T C R A S S S V S Y M H W  
 121 CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

BamHI  
 ~~~~~  
 Y Q Q K P G S S P K P W I Y A P S N L A
 181 TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATTT ATGCCCCATC CAACCTGGCT

S G V P A R F S G S G S G T S Y S L T I
 241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

S R V E A E D A A T Y Y C Q Q W S F N P
 301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

(Gly₄Ser)₃ Linker →
 P T F G A G T K L E L K G G G G S G G G
 361 CCCACGTTCTG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

2H7 V_H →
 G S G G G G S S Q A Y L Q Q S G A E L V
 421 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

R P G A S V K M S C K A S G Y T F T S Y
 481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

N M H W V K Q T P R Q G L E W I G A I Y
 541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

P G N G D T S Y N Q K F K G K A T L T V
 601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

D K S S S T A Y M Q L S S L T S E D S A
 661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

V Y F C A R V V Y Y S N S Y W Y F D V W
 721 GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG

Fig. 7B

human CD154/amino acid 108 →

Bcl/Bam hybrid site

BclI

781 G T G T T V T V S D P E N S F E M Q K G
GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAGAAAACA GCTTTGAAAT GCAAAAAGGT

BclI

~~~~~

841 D Q N P Q I A A H V I S E A S S K T T S  
GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAAATCT

901 V L Q W A E K G Y Y T M S N N L V T L E  
GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCCCTGGAA

961 N G K Q L T V K R Q G L Y Y I Y A Q V T  
AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

HindIII

~~~~~

1021 F C S N R E A S S Q A P F I A S L C L K
TTCTGTTCCA ATCGGGAAGC TTCGAGTCAA GCTCCATTTA TAGCCAGCCT CTGCCTAAAG

1081 S P G R F E R I L L R A A N T H S S A K
TCCCCCGGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

1141 P C G Q Q S I H L G G V F E L Q P G A S
CCTTGCGGGC AACAATCCAT TCACTTGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

NcoI

~~~~~

1201 V F V N V T D P S Q V S H G T G F T S F  
GTGTTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

XhoI

XbaI

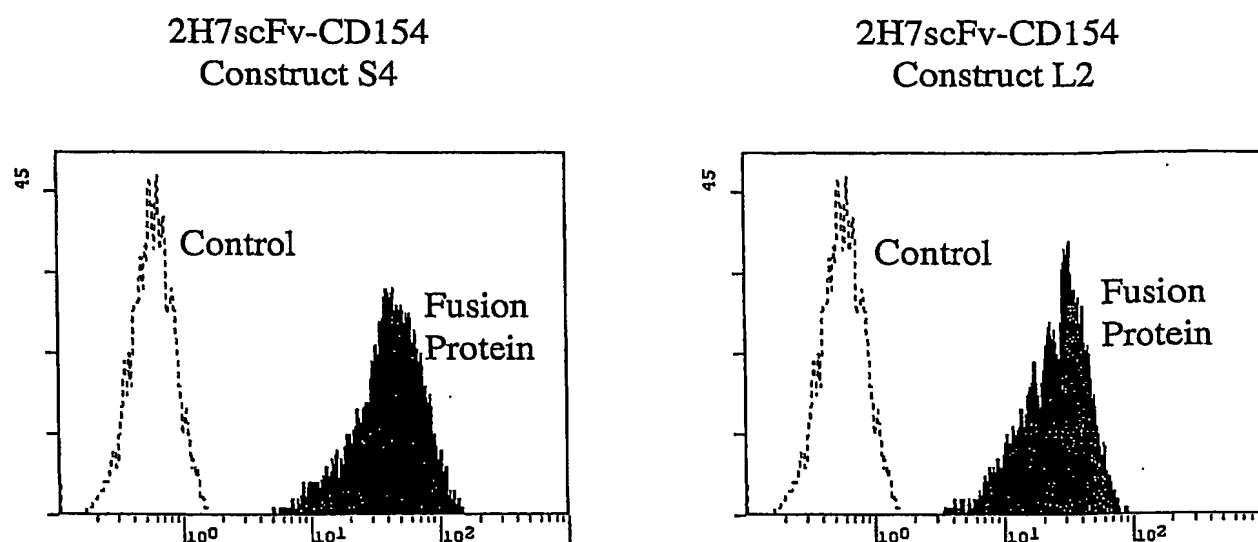
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1261 G L L K L E \* \* S R  
GGCTTACTCA AACTCGAGTG ATAATCTAGA

Fig. 8

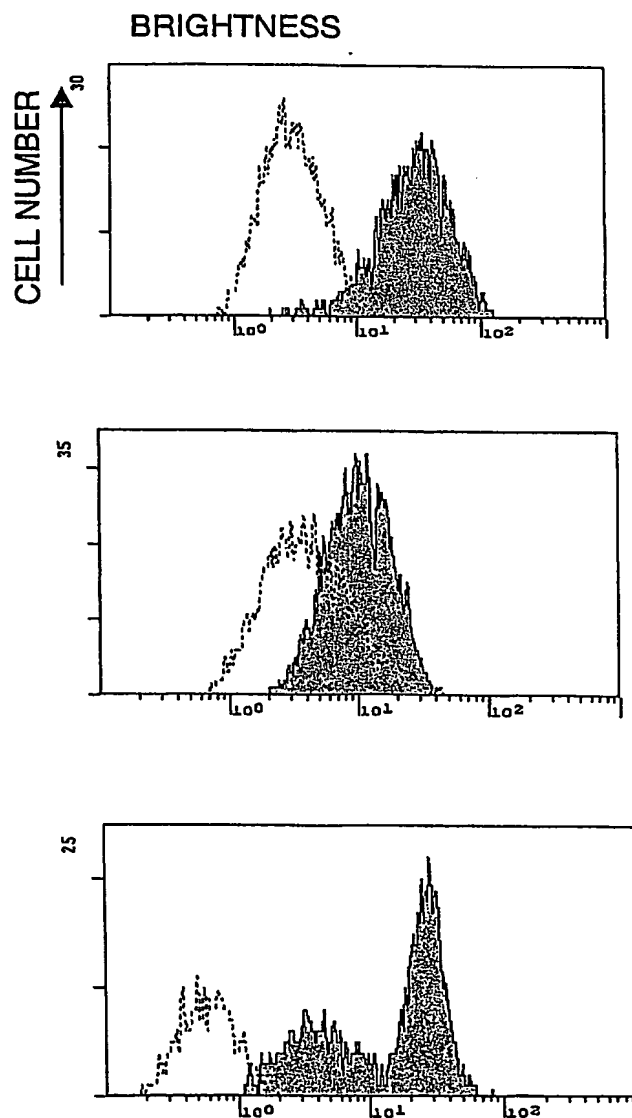
Simultaneous Binding of 2H7scFv-CD154  
Fusion Proteins to CD20 and CD40



CD20 CHO cell targets + (control or fusion protein)  
+ Biotin-CD40Ig + PE-SA

Fig. 9

Induction of Apoptosis Measured by Binding of Annexin V after incubation with 2H7scFv-CD154



.....control supernatant    — 2H7scFv-CD154 supernatant

Fig. 10

Proliferation of T51 B Cell Line After Incubation with 2H7  
scFv-CD154 S4 or 2H7 scFv-CD154 L2 Constructs

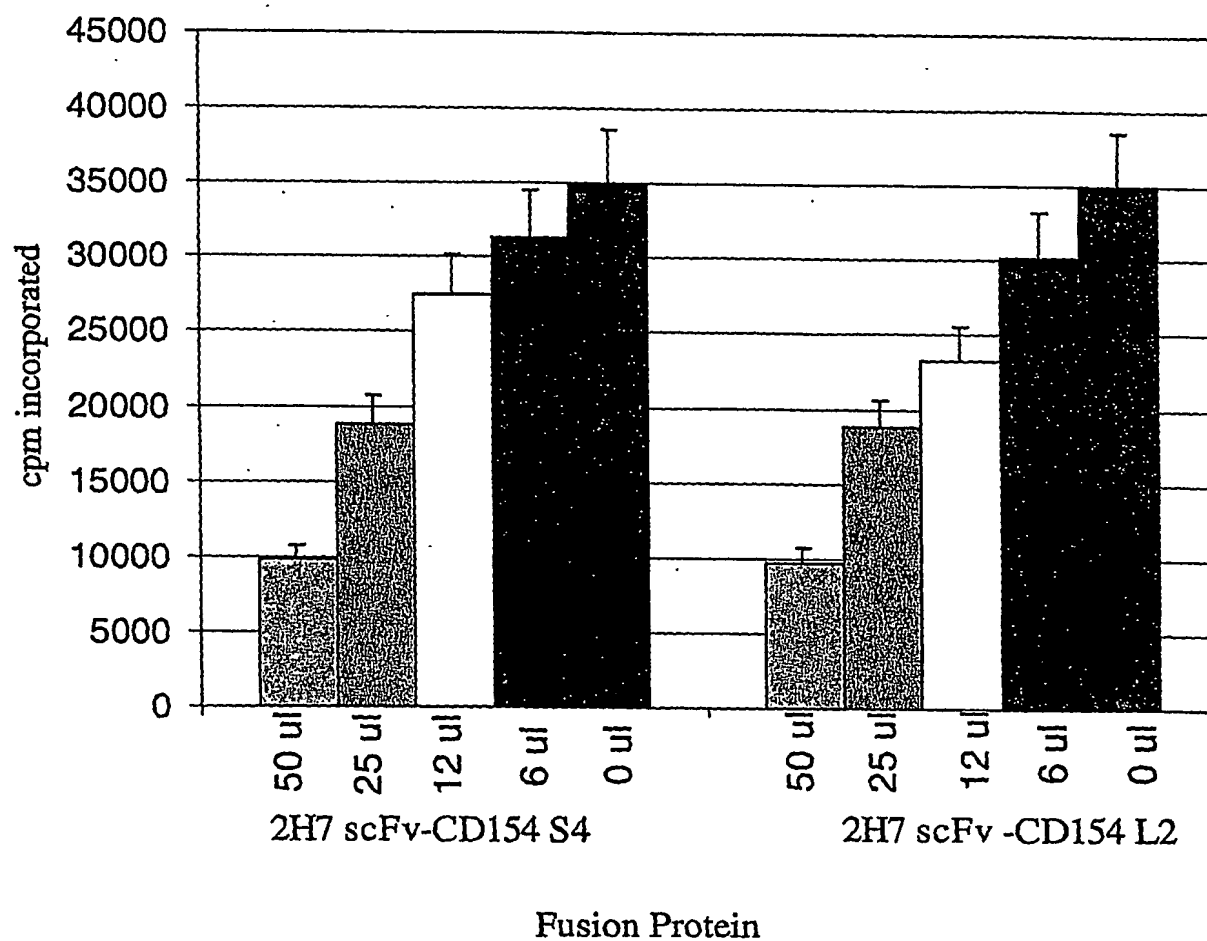


Fig. 11

## Schematic Representation of 2H7 scFvIg Constructs

2H7 scFvIgG (SSS-S)H WCH2 WCH3

OR 2H7 scFvIgG1 (SSS-S)H P238SCH2 WCH3 : 2H7 scFv

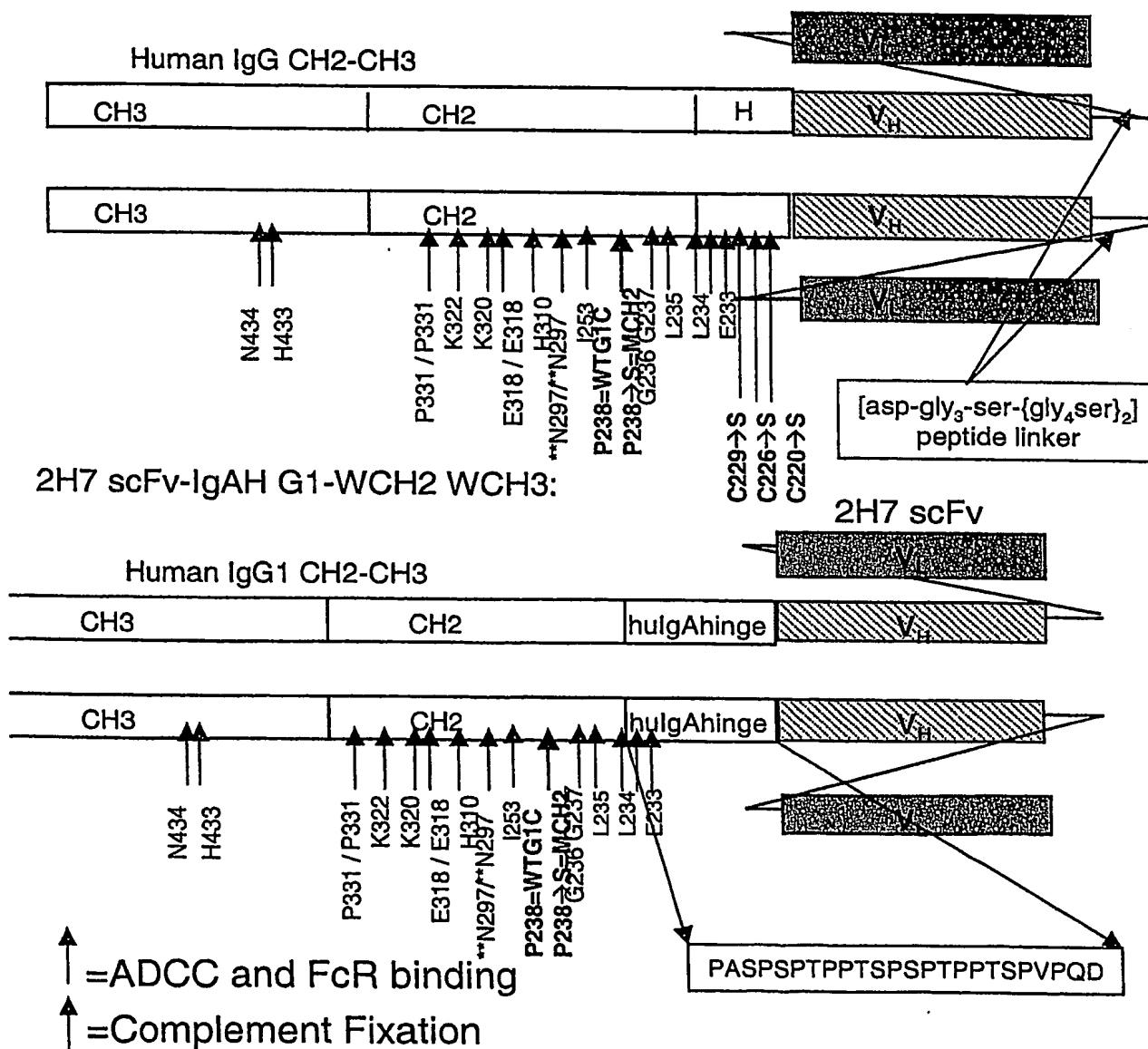


Fig. 12

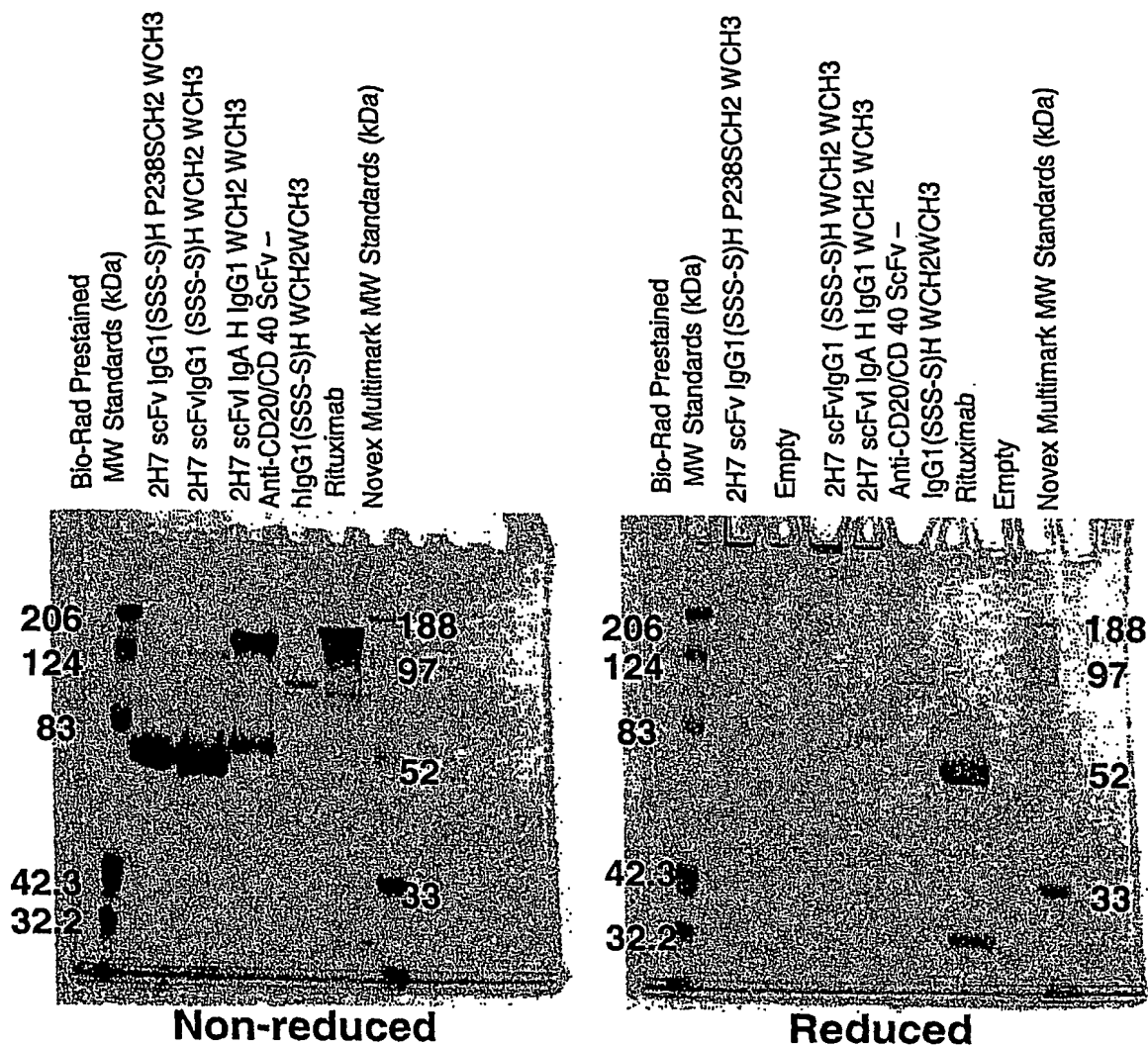


Figure 12: SDS-PAGE Analysis of CytoxB Derivatives. Purified fusion protein derivatives of CytoxB-scFvIg molecules and Rituximab were resuspended in SDS sample buffer, boiled, loaded onto 10% Novex Tris-Bis gels (Invitrogen, San Diego, CA) and subjected to nonreducing (left panel) or reducing (right panel) SDS-PAGE electrophoresis at 175 volts. Two different molecular weight markers, BioRad prestained markers, and Novex Multimark molecular weight markers were also loaded onto each gel and the approximate size in kDa of each marker band is indicated along each side of the photographed gels. Gels were stained in Coomassie Blue stain and photographed with a SONY Mavica Digital camera. The mutant hinge forms of 2H7 scFvIgG1 migrate at approximately 70 kDa under both nonreducing and reducing conditions, indicating that these molecules are monomeric rather than dimeric in structure. The IgA hinge form of 2H7scFvIg migrates at approximately 75 kDa under reducing conditions, but migrates predominately as a dimer of 140 kDa with a fraction of the protein migrating at 75 kDa under nonreducing conditions. Under nonreducing conditions, rituximab migrates as a diffuse band of between 150 and 200 kDa. The heavy and light chains resolve into separate bands of approximately 32 and 50 kDa when rituximab is reduced and subjected to SDS-PAGE.



Fig. 13

## ADCC Activity of CytosB (2H7 scFvIg) Constructs.

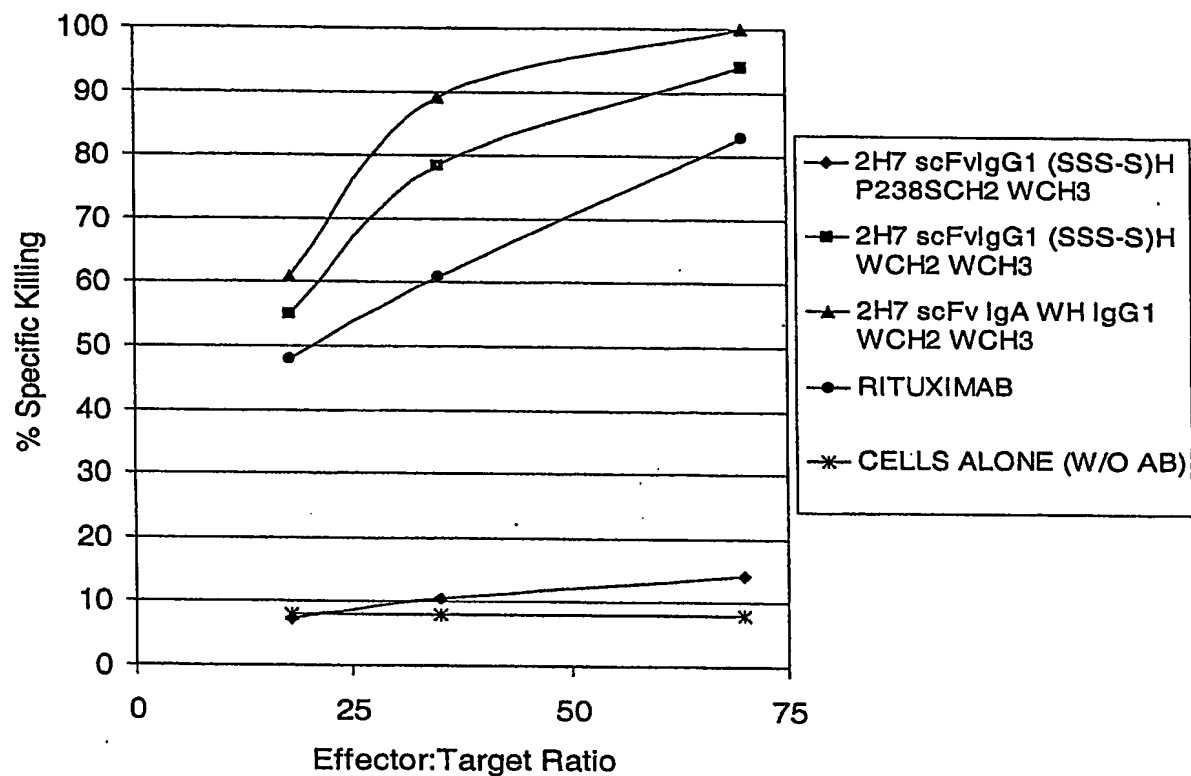


Figure 13: ADCC Activity of CytosB Derivatives Compared to Rituximab. ADCC activity of CytosB Derivatives or Rituximab was measured *in vitro* against BJAB B lymphoma cell line as target and using fresh human PBMC as effector cells. Effector to target ratios were varied as follows: 70:1, 35:1, and 18:1, with the number of BJAB cells per well remaining constant but varying the number of PBMC. Bjab cells were labeled for 2 hours with  $^{51}\text{Cr}$  and aliquoted at a cell density of  $5 \times 10^4$  cells/well to each well of flat-bottom 96 well plates. Purified fusion proteins or rituximab were added at a concentration of 10 mg/ml, and PBMC were added at  $9 \times 10^5$  cells /well (18:1),  $1.8 \times 10^6$  cells/well (35:1), or  $3.6 \times 10^6$  cells/well (70:1), in a final volume of 200  $\mu\text{l}$ . Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells. Reactions were incubated for 4 hours, and 100  $\mu\text{l}$  culture supernatant harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released on a Packard Top Count NXT Microplate Scintillation Counter.

Fig. 14

## CDC of Cytox B (2H7 scFvIg) Constructs

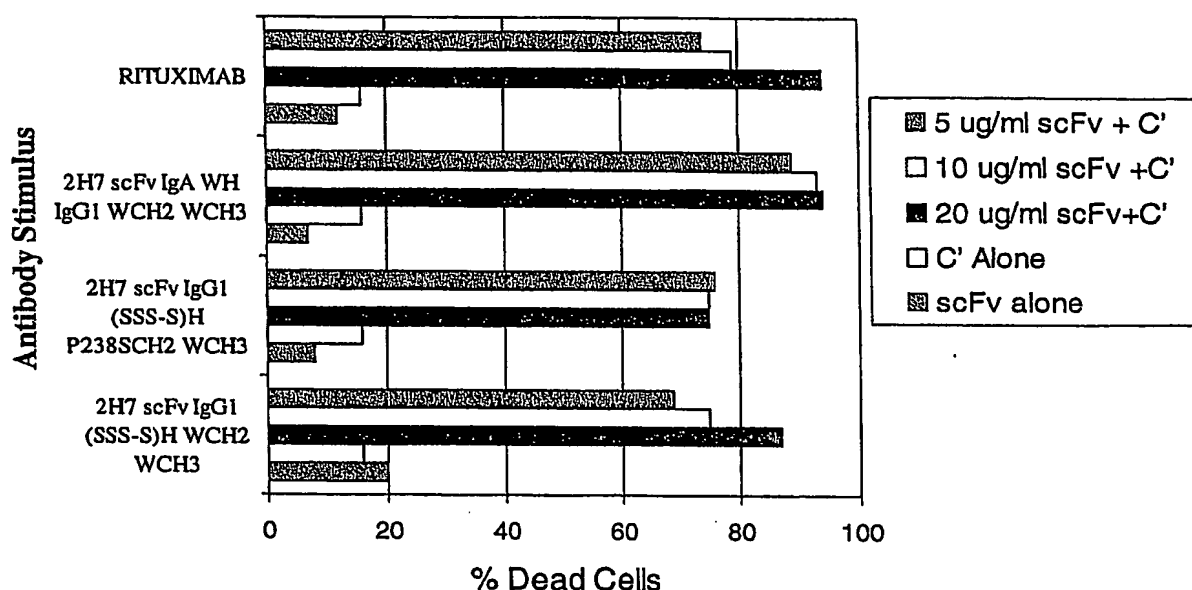
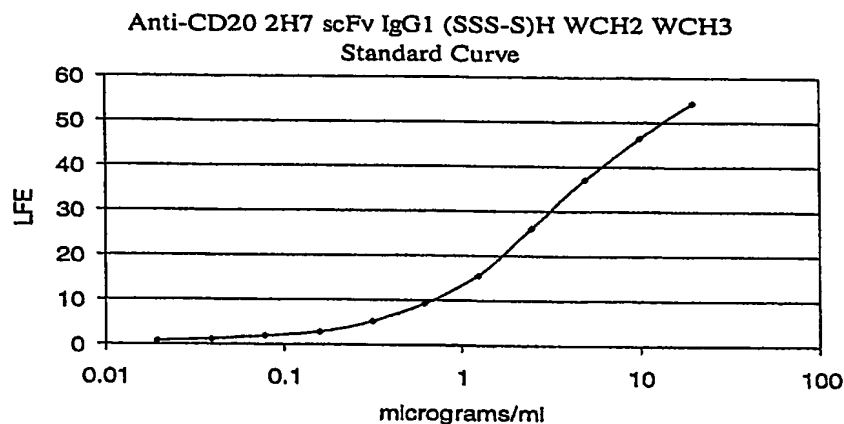


Figure 14: Complement Dependent Cytotoxicity (CDC) Activity of CytoxB Derivatives Compared to Rituximab. 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3, 2H7 scFvIgG1 (SSS-S)H WCH2 WCH3, and 2H7scFv IgA WH IgG1 WCH2 WCH3 derivatives and Rituximab were compared for their ability to mediate complement dependent cytotoxicity. Rabbit complement (Pel-Freez) was diluted 1:10 and added to BJAB cells along with dilutions of each antibody derivative (20  $\mu$ g/ml, 10  $\mu$ g/ml, and 5  $\mu$ g/ml). Controls were also included without addition of complement (C') or scFv derivative. Reactions were allowed to continue for 1 hour, and cells from each well were then stained with trypan blue and the cell viability counted using a hemacytometer. Data is graphed as % of dead cells/total cells counted for each condition assayed.

Fig. 15

# 2H7 (anti-CD20) scFv IgG1 (SSS-S)H WCH2 WCH3 In Vivo Half Life



## Macaque A99314

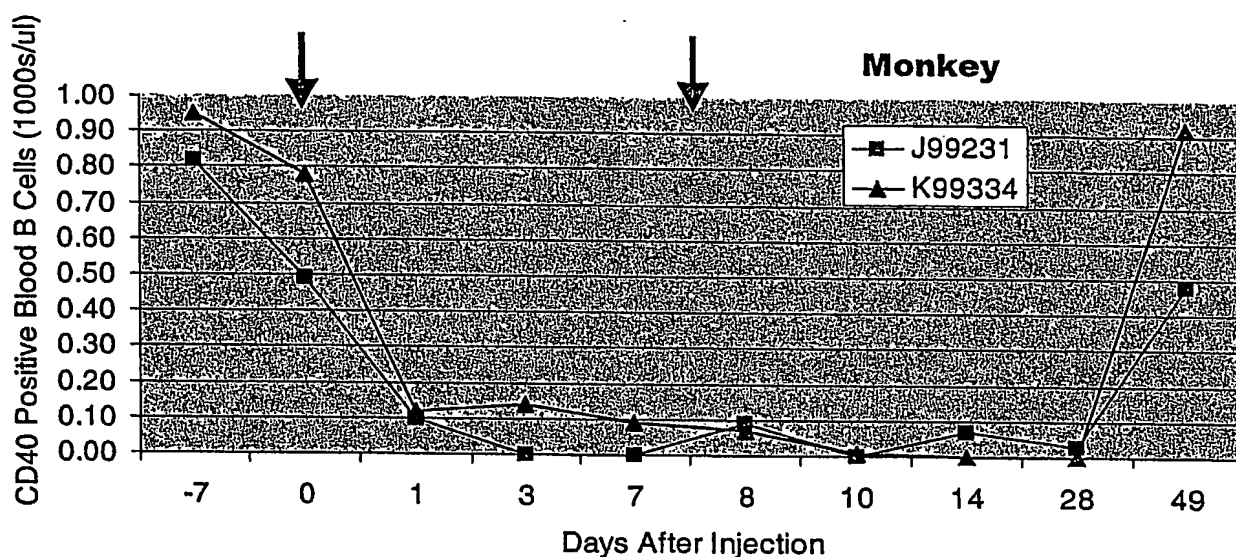
|              | Day | Binding intensity (LFE)<br>@ 1:50 dilution of serum | estimated<br>concentration (µg/ml) |
|--------------|-----|-----------------------------------------------------|------------------------------------|
| Injection #1 | -7  | 0.213                                               | <0.1                               |
|              | 0   | 0.227                                               | <0.1                               |
|              | 1   | 7.79                                                | 25.1                               |
| Injection #2 | 3   | 5.51                                                | 15.6                               |
|              | 7   | 3.37                                                | 9.4                                |
|              | 8   | 11.33                                               | 41.7                               |
|              | 10  | 5.45                                                | 15.4                               |
|              | 14  | 0.27                                                | <0.1                               |

## Macaque F98081

|              | Day | Binding intensity (LFE)<br>@ 1:50 dilution of serum | estimated<br>concentration (µg/ml) |
|--------------|-----|-----------------------------------------------------|------------------------------------|
| Injection #1 | -7  | 0.208                                               | <0.1                               |
|              | 0   | 0.219                                               | <0.1                               |
|              | 1   | 6.73                                                | 21.9                               |
| Injection #2 | 3   | 6.14                                                | 19.3                               |
|              | 7   | 3.04                                                | 8.7                                |
|              | 8   | 9.83                                                | 33.8                               |
|              | 10  | 4.77                                                | 14.4                               |
|              | 14  | 0.231                                               | <0.1                               |

Fig. 16

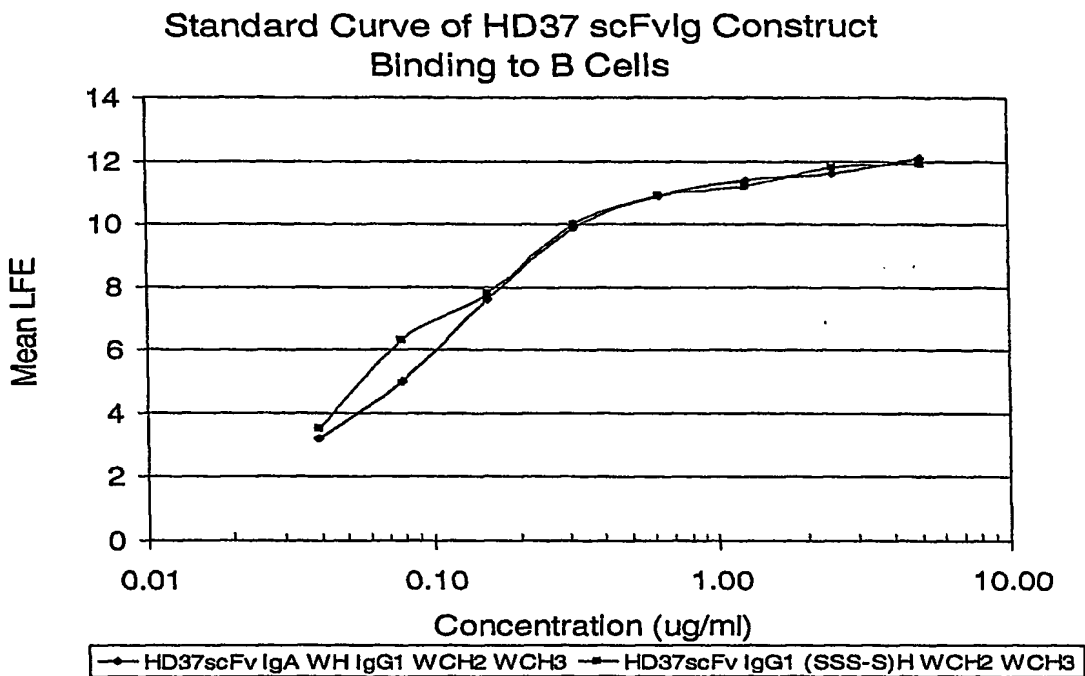
B Cell Depletion in macaques mediated by Cytos B20  
(2H7 scFv IgG1 (SSS-S)H WCH2 WCH3) Construct



- CytosB20 injections of 6mg/kg yields 3 week B-cell depletion
- 3-4 day half-life *in vivo*
- CD20 saturation in lymph node B-cells at d14
- No first dose effects
- No anti-chimeric antibody development

Fig. 17

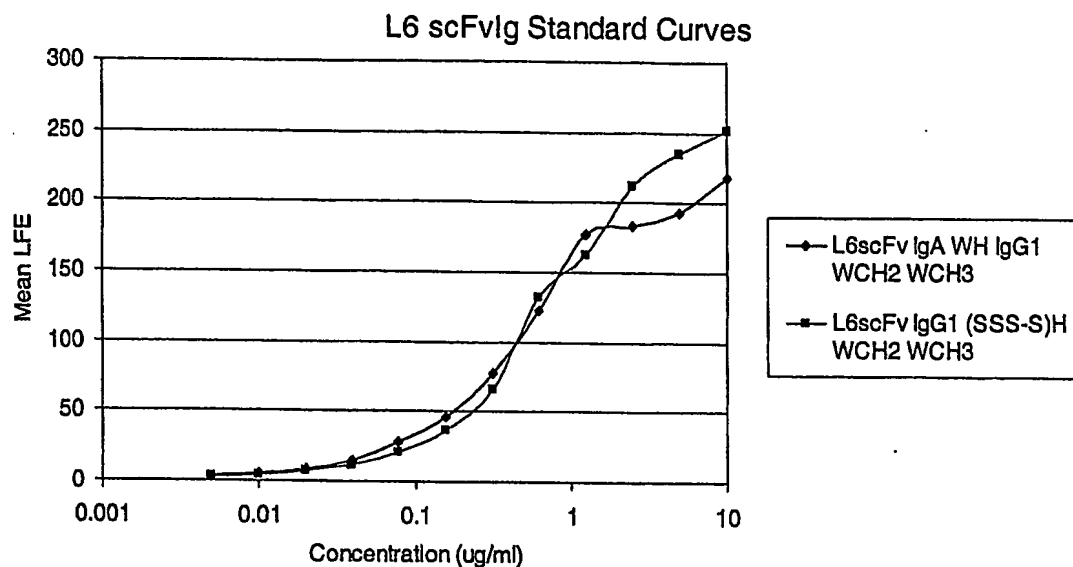
### Production Levels of HD37 scFvIg Constructs by CHO Cell Lines



| Clone/Isolate           | Mean LFE at 1:100 | Estimated Concentration |
|-------------------------|-------------------|-------------------------|
| Bulk HD37 scFv          |                   |                         |
| IgA WH IgG1 WCH2 WCH3   | 11.2              | > 60 ug/ml              |
| 1B2                     | 10.4              | >50 ug/ml               |
| 6C5                     | 10.5              | >50 ug/ml               |
| 4B1                     | 8.6               | >40 ug/ml               |
| Bulk HD37 scFv          |                   |                         |
| IgG1 (SSS-S)H WCH2 WCH3 | 10.9              | > 50 ug/ml              |
| 2G8                     | 10.6              | > 50 ug/ml              |
| 3F3                     | 8.3               | >40 ug/ml               |
| 3D9                     | 11.1              | > 60 ug/ml              |

Fig. 18

## Production of L6 scFvIg constructs by CHO Cells



| Construct | Mean LFE 1:20 | Estimated Concentration |
|-----------|---------------|-------------------------|
|-----------|---------------|-------------------------|

|                                                        |      |            |
|--------------------------------------------------------|------|------------|
| L6scFv IgA WH<br>IgG1 WCH2 WCH3<br>unamplified CHO sup | 51.1 | 6.25 ug/ml |
|--------------------------------------------------------|------|------------|

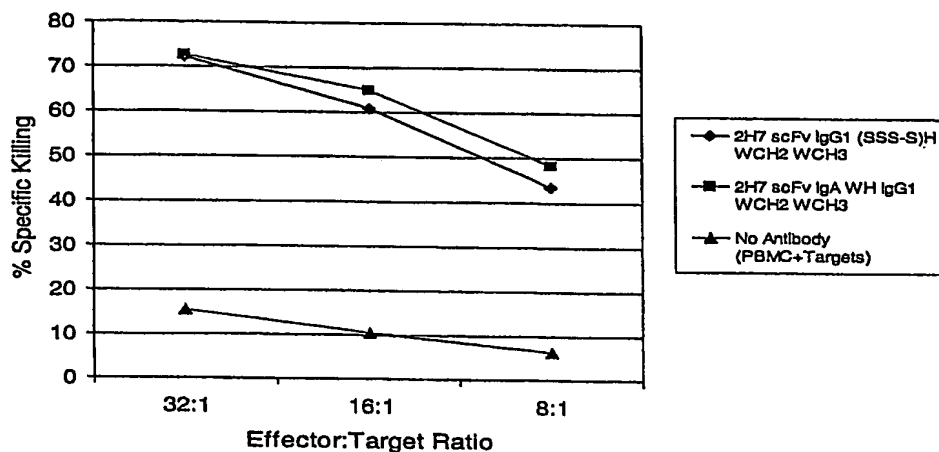
|                                                         |      |           |
|---------------------------------------------------------|------|-----------|
| L6scFv IgG1(SSS-S)H<br>WCH2 WCH3<br>unamplified CHO sup | 23.0 | 3.2 ug/ml |
|---------------------------------------------------------|------|-----------|

**Fig. 19**

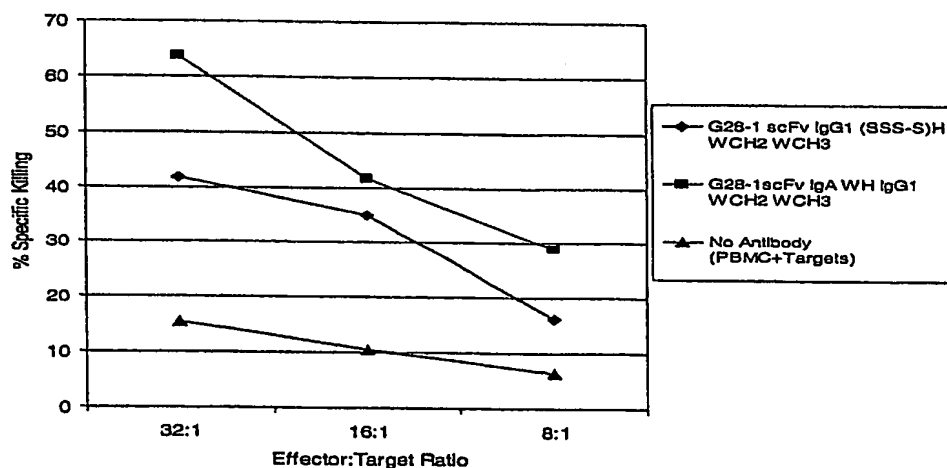
# ADCC Activity of 2H7 scFvIg, G28-1 scFvIg, and HD37 scFvIg Constructs

## ADCC Activity of scFvs Targeted to B Cell Antigens

### A. 2H7 (anti-CD20) scFv constructs



### B. G28-1 (anti-CD37) scFv constructs



### C. HD37 (anti-CD19) scFv constructs

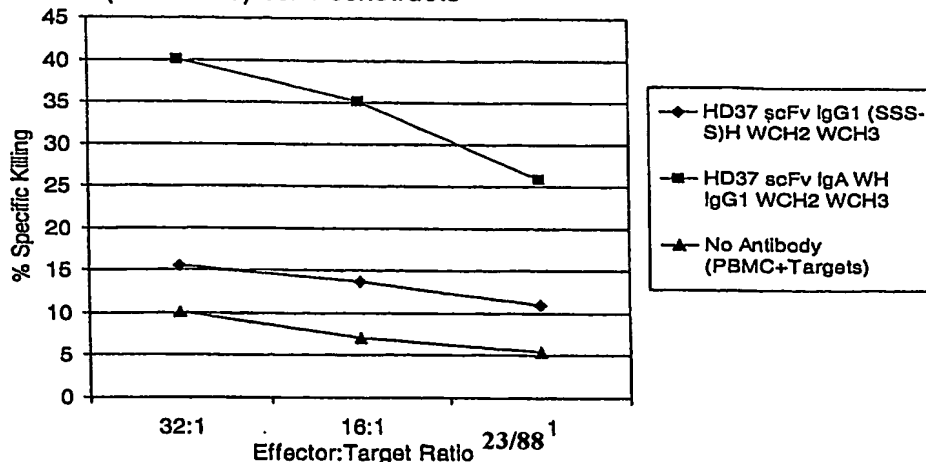


Fig. 20

## ADCC Activity of L6 scFvIg Constructs

ADCC Activity of L6scFvIg Constructs with 2981 Targets

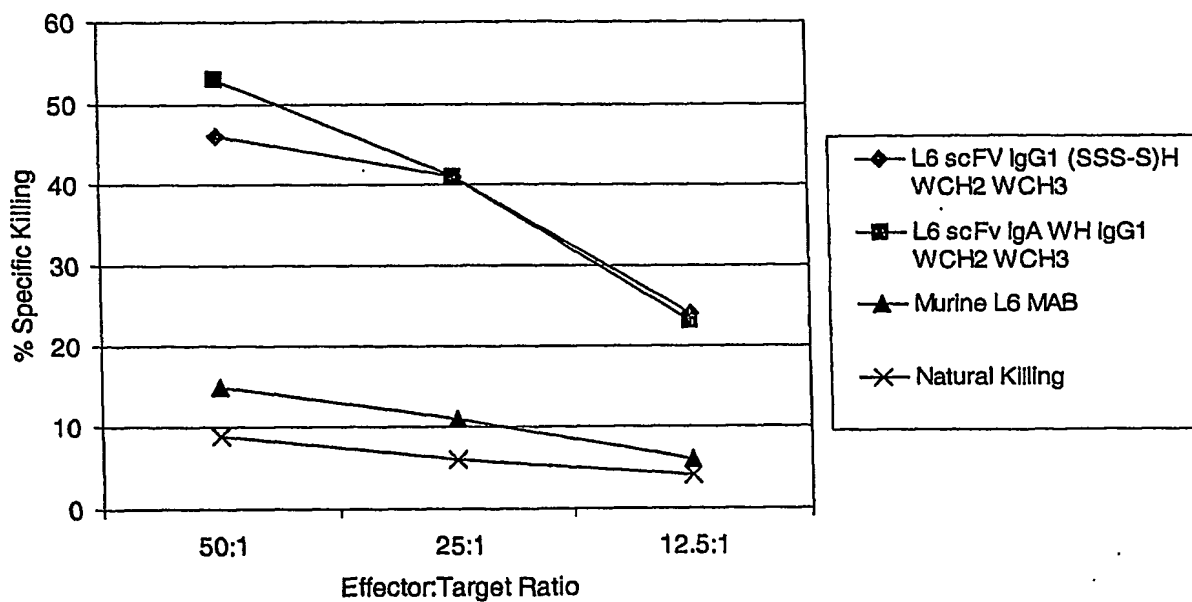




Fig. 21

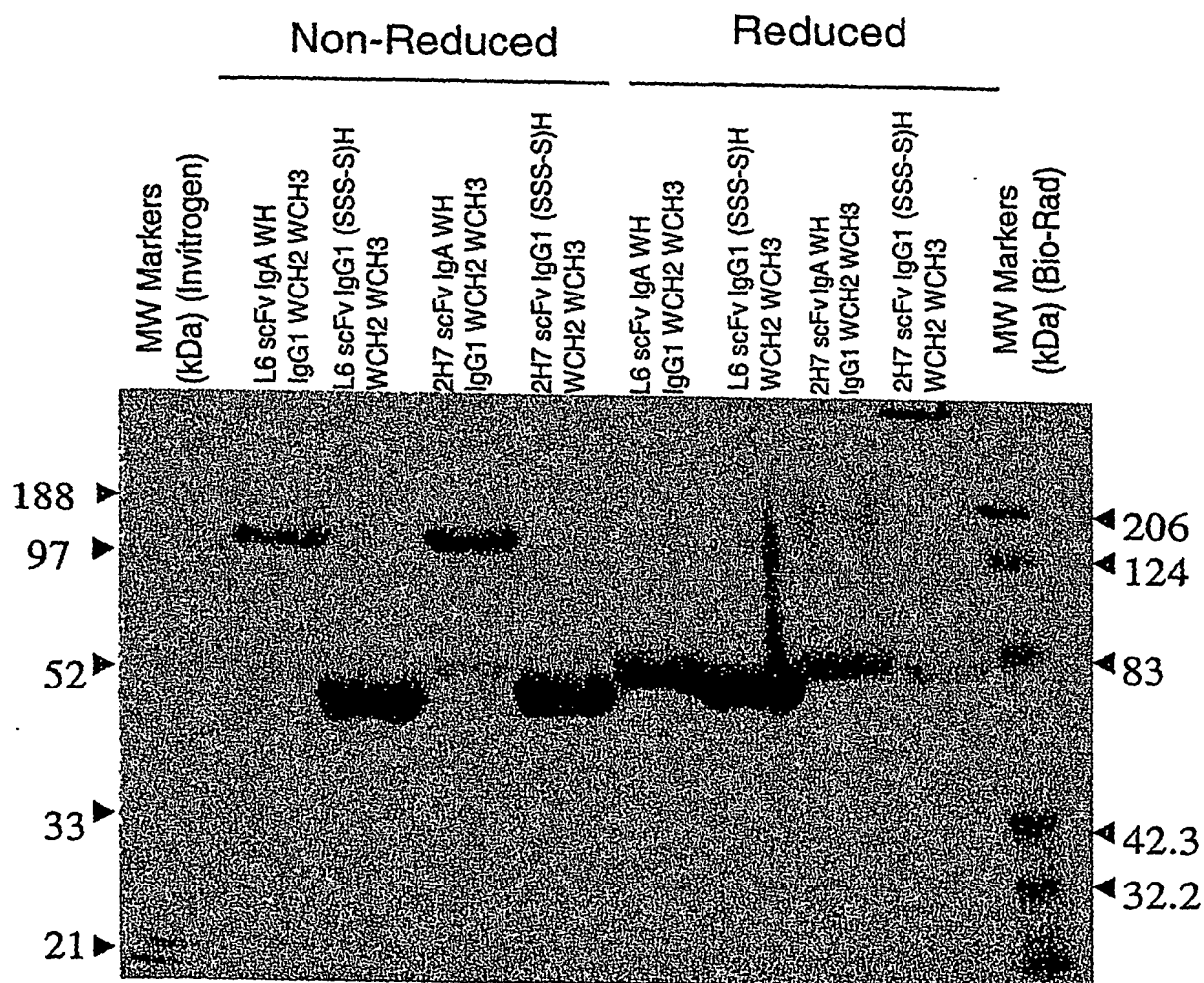
SDS-PAGE Analysis of L6 and 2H7  
scFvIg Fusion Proteins.

Fig. 22

# SDS-PAGE Analysis of G28-1 and HD37 scFvlg Constructs.

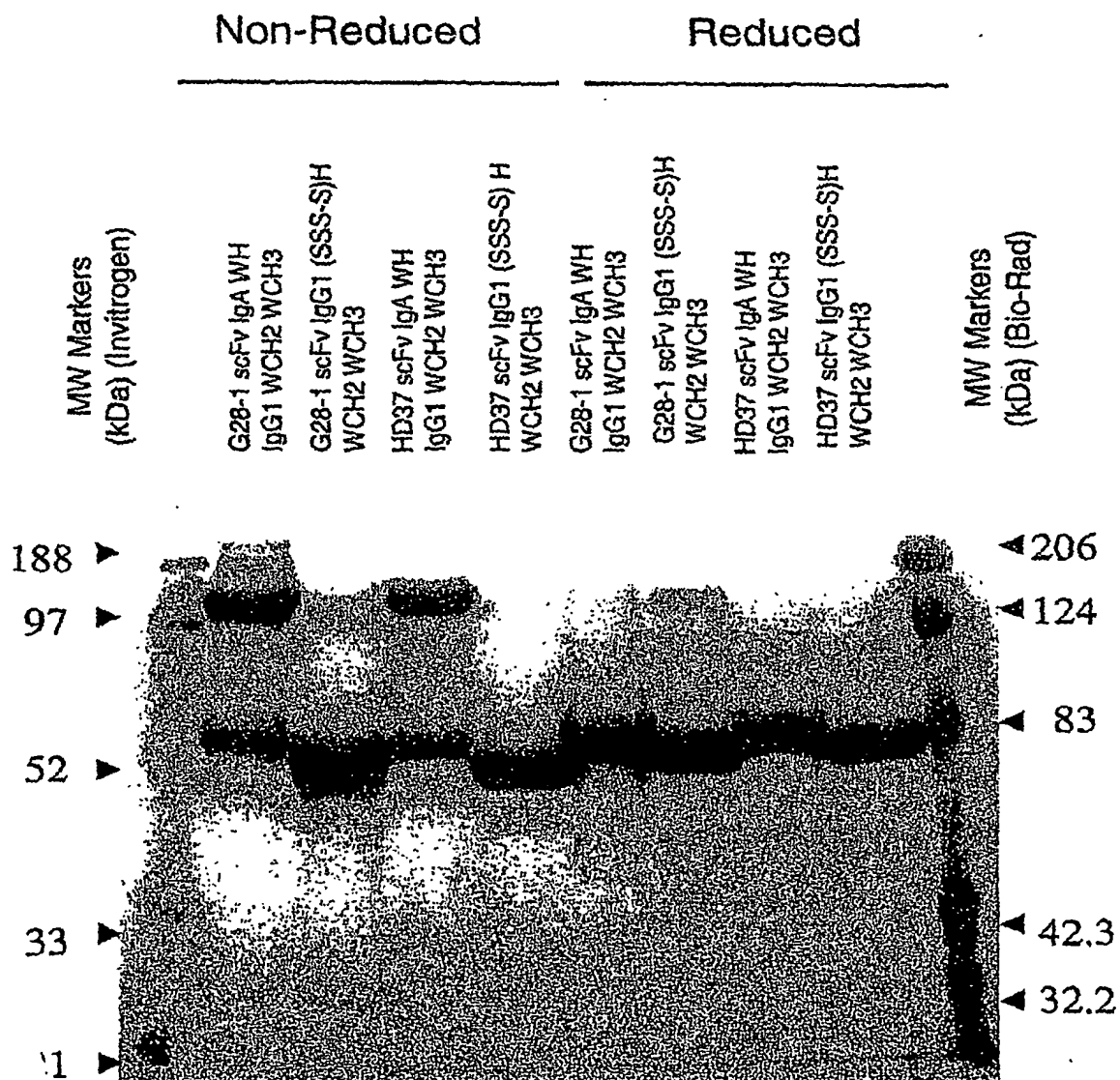


Fig. 23

Sequence alignment of human and llama Fc regions.

HINGE

CH2→

|          |                             |                                                       |
|----------|-----------------------------|-------------------------------------------------------|
| an IgG1: | DQEPKSCDKT-----HTCPPC       | PAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG |
| ma IgG2: | DQEPKTPKPQPPQPPNPPTTESKCPKC | PAPPELLGGPSVFIFPPKPKDVLISIGRPEVTCVVVDVGQEDPEVSFNWYIDG |
| ma IgG1: | --EPHGG-----CTCPQC          | PAPELPGGPSVFVFPPKPKDVLISIGRPEVTCVVVDVGKEDPEVNFNWYIDG  |
| ma IgG3: | --AHHSEDPT-----SKCPKC       | PGPELLGGPTVFIFPPKAKDVLISITRKPEVTCIWWTWVKKTLRSSSSWSVDD |

VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT  
 TAEVRANTRPKEEQFNSTYRVVSVLPIQHQQDWLTGKEFKCKVNNKALPAPIEKTISKAKGQTPREPQVYTLAPHREELAKDTVSVT  
 VEVRTANTKPKKEEQFNSTYRVVSVLPIQHQQDWLTGKEFKCKVNNKALPAPIERTISKAKGQTPREPQVYTLAPHREELAKDTVSVT  
 TEVHTAETKPKKEEQFNSTYRVVSVLPIQHQQDWLTGKEFKCKVNNKALPAPIERTISKAKGQTPREPQVYTLAPHREELAKDTVSVT

CLVKGFFPSDIAVEWESNGQPEN--NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGK  
 CLVKGFFYPPDINVEWQRNGQPESXGTYATTPQLDNDGTYFLXSKXSVGKNTWQQGETFTCVVMHEALHNHYTQKSITQSSGK  
 CLVKGFFPADINVEWQRNGQPESEGTYANTPPQLDNDGTYFLYSRLSVGKNTWQRGETLTGVVMHEALHNHYTQKSITQSSGK  
 CLVKGFFPADINVEWQRNGQPESEGTYANTPPQLDNDGTYFLYSKLSVGKNTWQQGEVFTCVVMHEALHNHSTQKSITQSSGK

Fig. 24

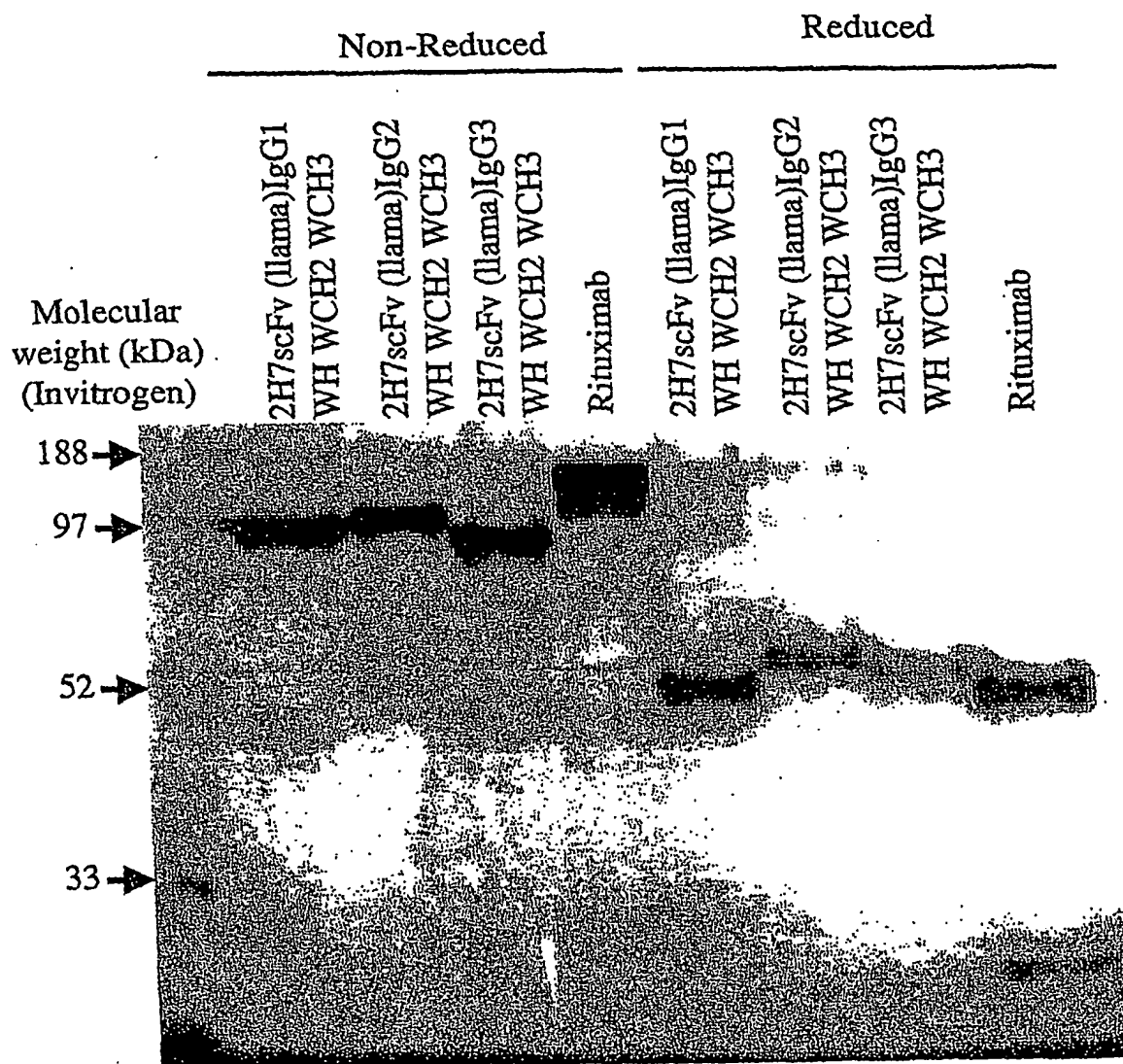


Fig. 25

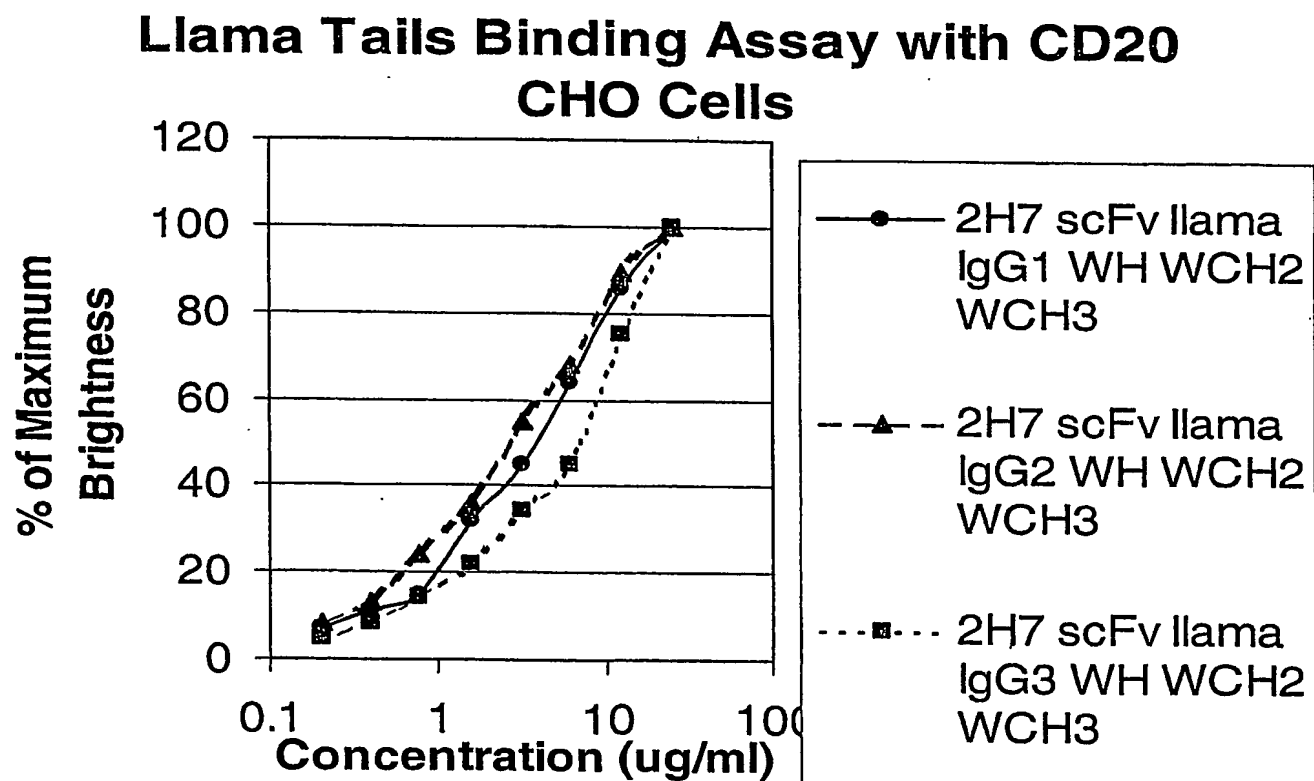


Fig. 26

2H7 scFvIg Llama Tails binding Assay with CD20 CHO Cells

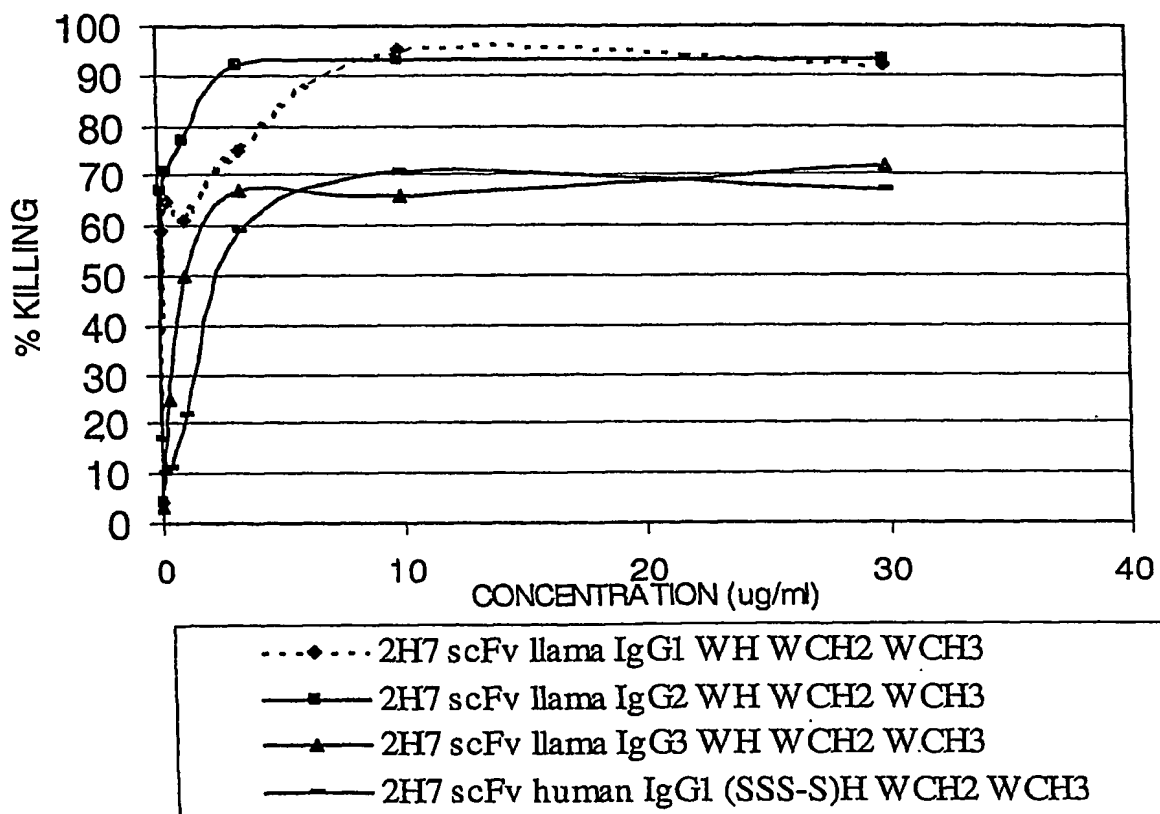


Fig. 27

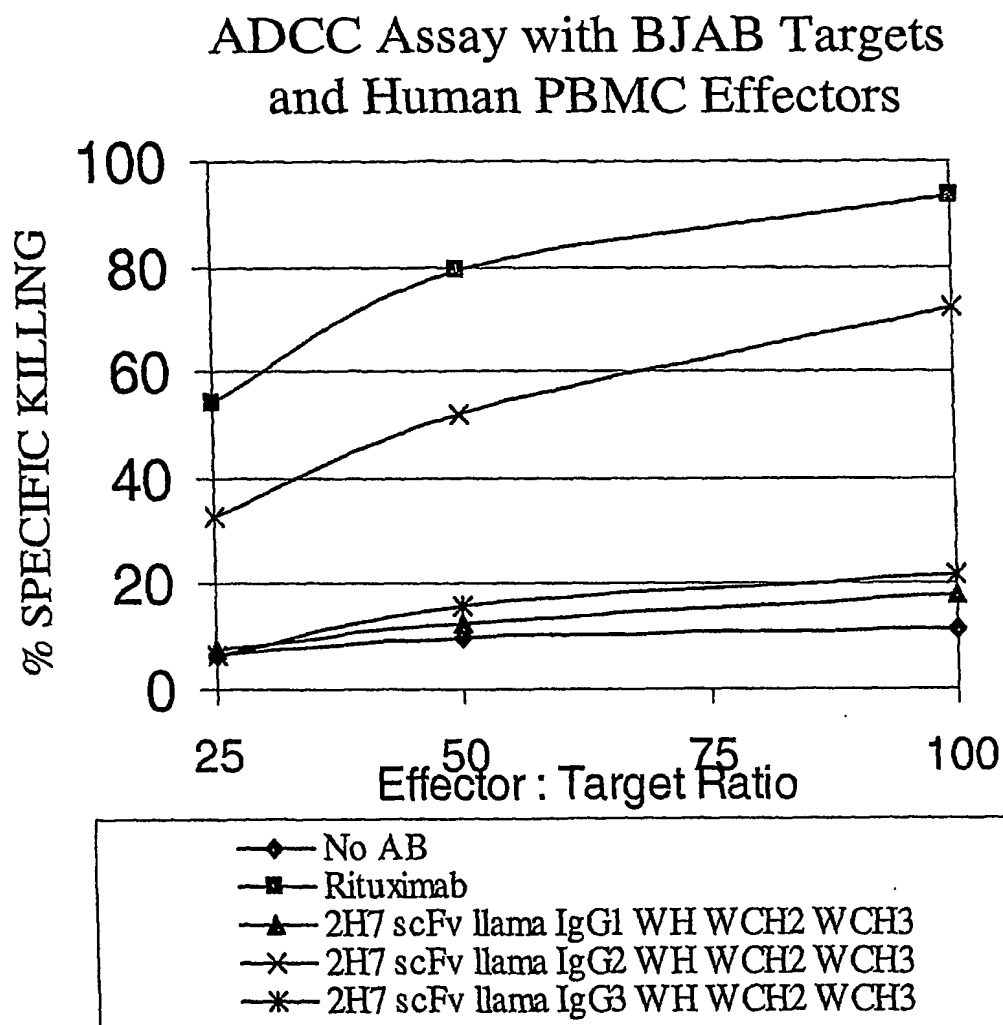


Fig. 28

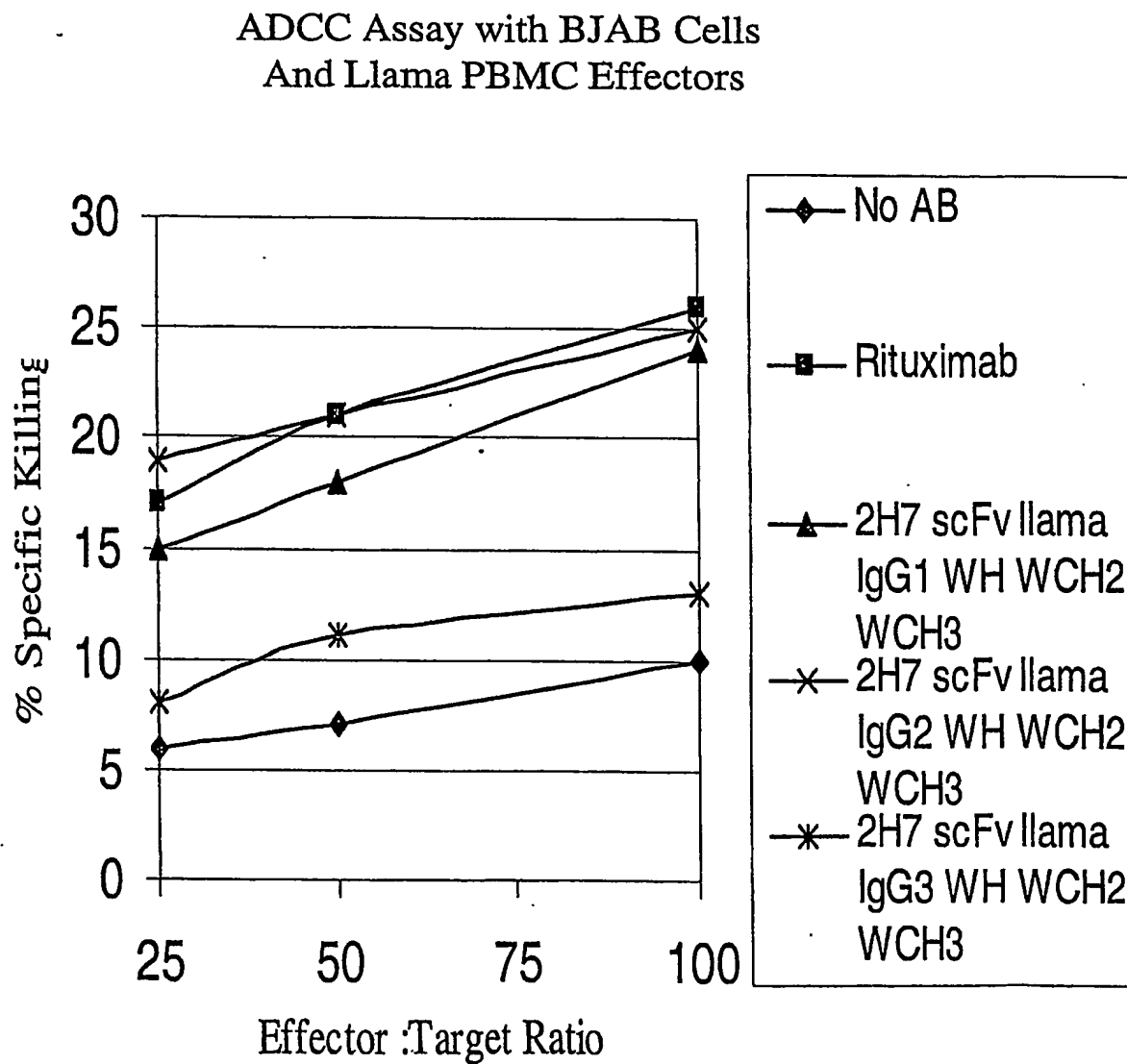




Fig. 29

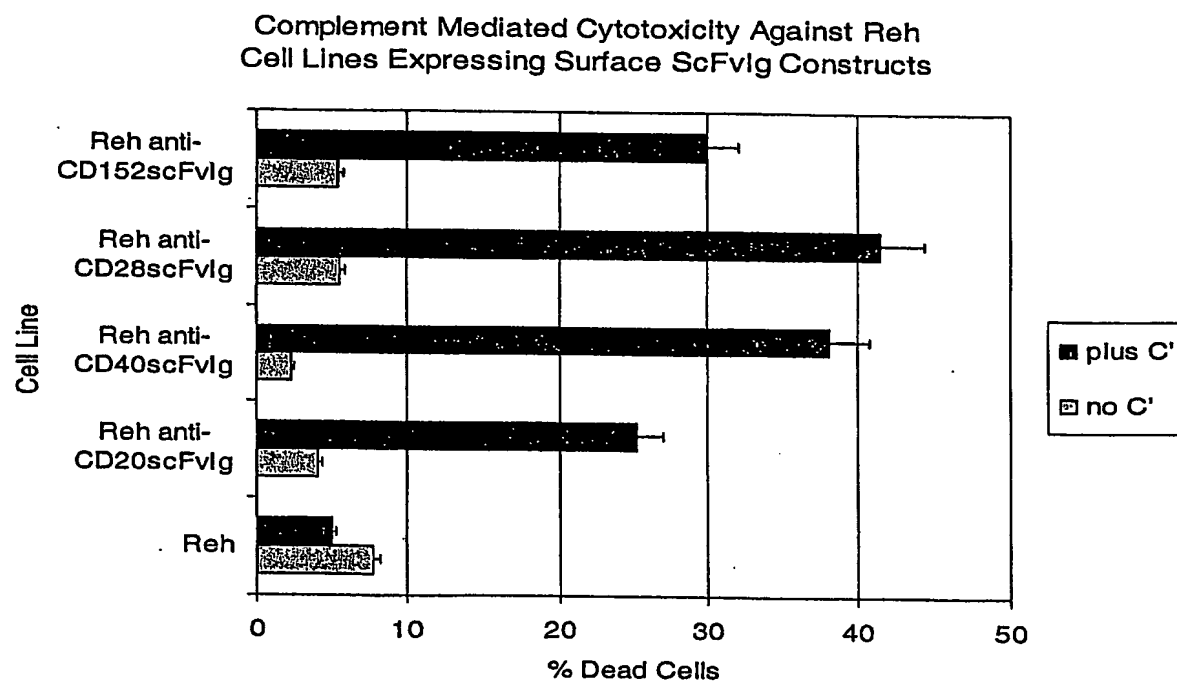


Fig. 30

## ADCC Activity of Cell Surface Expressed ScFvlg Constructs

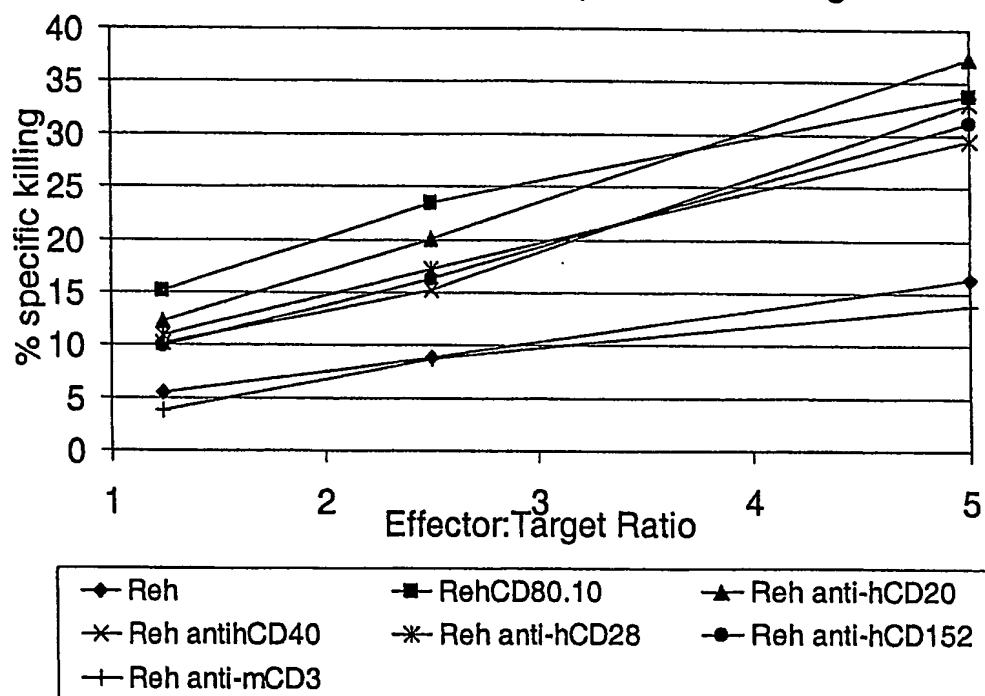


Fig. 31

## Ig Constructs and Nomenclature:

| Name Identifier                        | Hinge Sequence               | CH2 Sequence                     | CH3 Sequence                                |
|----------------------------------------|------------------------------|----------------------------------|---------------------------------------------|
| hIgG1 (CCC-P)H<br>WCH2 WCH3            | IgG1 WT Hinge<br>(CCC-P)     | Wild Type CH2                    | Wild Type CH3                               |
| hIgG1 (SSS-S)H<br>WCH2 WCH3            | IgG1 Mutant Hinge<br>(SSS-S) | Wild type CH2<br>(IgG1)          | Wild type CH3 (IgG1)                        |
| VH L11S<br>hIgG1 (SSS-S)H<br>WCH2 WCH3 | IgG1 Mutant Hinge<br>(SSS-S) | Wild type CH2<br>(IgG1)          | Wild type CH3 (IgG1)                        |
| IgG1 (SSC-S)H<br>WCH2 WCH3             | IgG1 Mutant Hinge<br>(SSC-S) | Wild type CH2<br>(IgG1)          | Wild type CH3 (IgG1)                        |
| IgG1 (SCS-S)H<br>WCH2 WCH3             | IgG1 Mutant Hinge<br>(SCS-S) | Wild type CH2<br>(IgG1)          | Wild type CH3 (IgG1)                        |
| IgG1 (CSS-S)H<br>WCH2 WCH3             | IgG1 Mutant Hinge<br>(CSS-S) | Wild type CH2<br>(IgG1)          | Wild type CH3 (IgG1)                        |
| IgG1 (SSS-S)H<br>P238S CH2 WCH3        | IgG1 Mutant Hinge<br>(SSS-S) | Mutant CH2 (IgG1)<br>Pro→Ser 238 | Wild type CH3 (IgG1)                        |
| IgA WH hIgG1<br>WCH2 WCH3              | IgA Hinge                    | Wild type CH2<br>(IgG1)          | Wild type CH3 (IgG1)                        |
| IgA WH IgA<br>WCH2 WCH3                | IgA Hinge                    | Wild type CH2 (IgA)              | Wild type CH3 (IgA)                         |
| IgA WH IgA<br>WCH2 T4CH3               | IgA Hinge                    | Wild type CH2 (IgA)              | Truncated CH3 (IgA)<br>Missing 4 aa at COOH |

Fig. 32

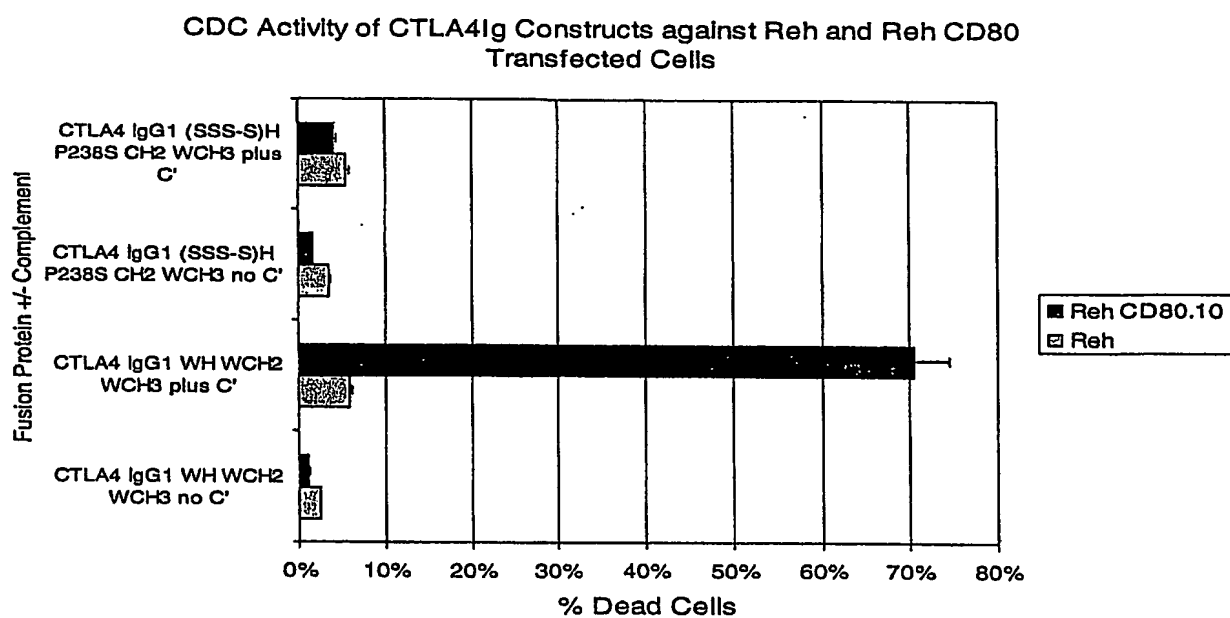


Fig. 33

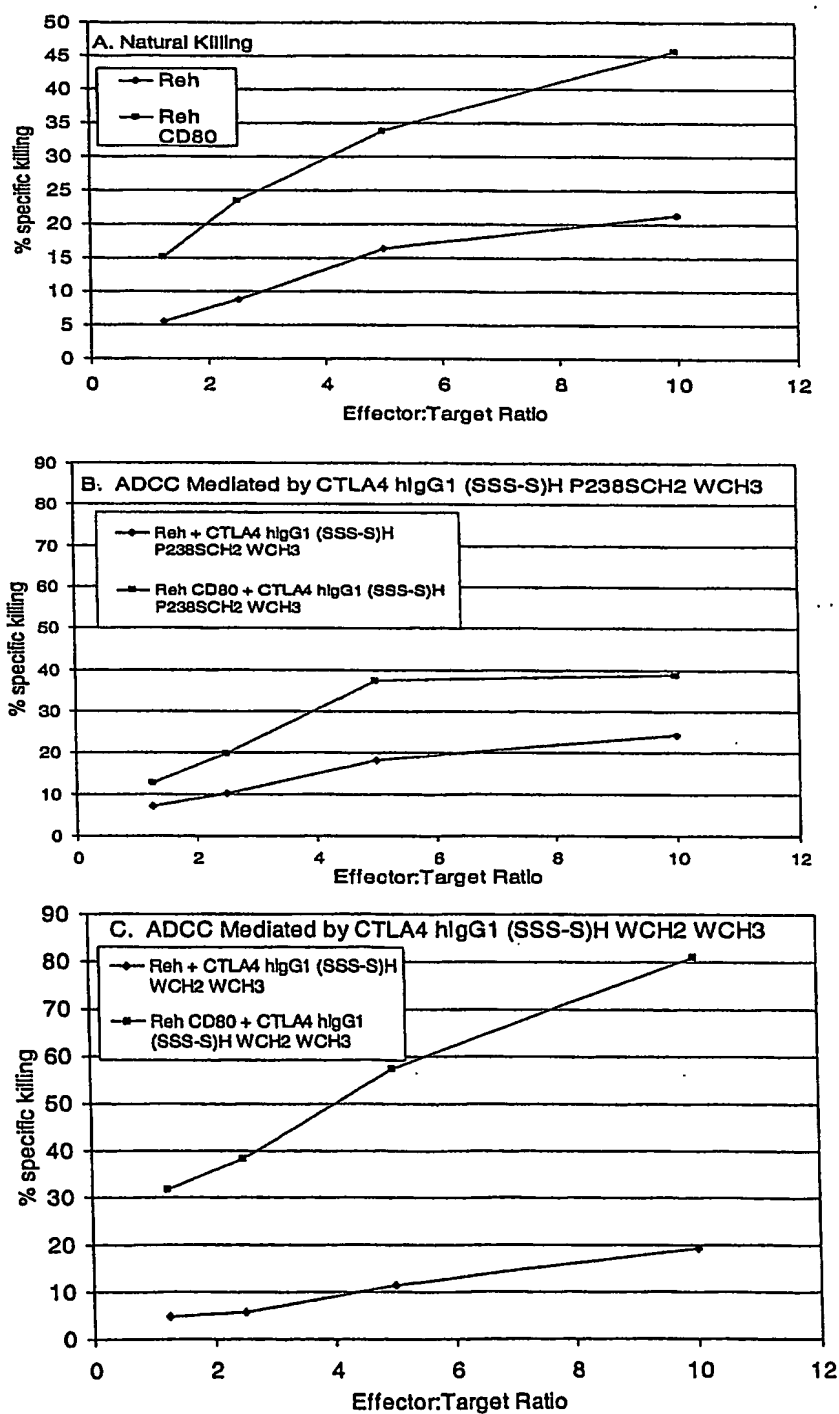


Fig. 34

Binding of 2H7 scFvIg Constructs  
with Alternative Tails to CD20 CHO Cells

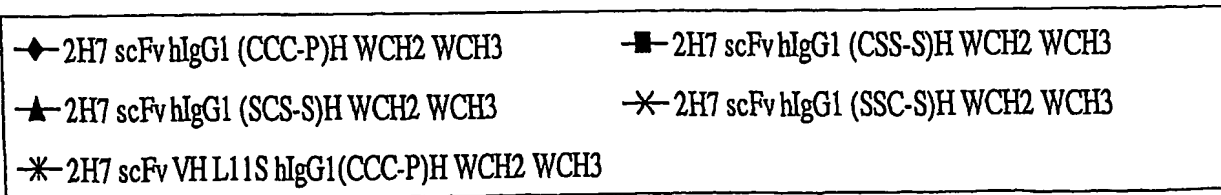
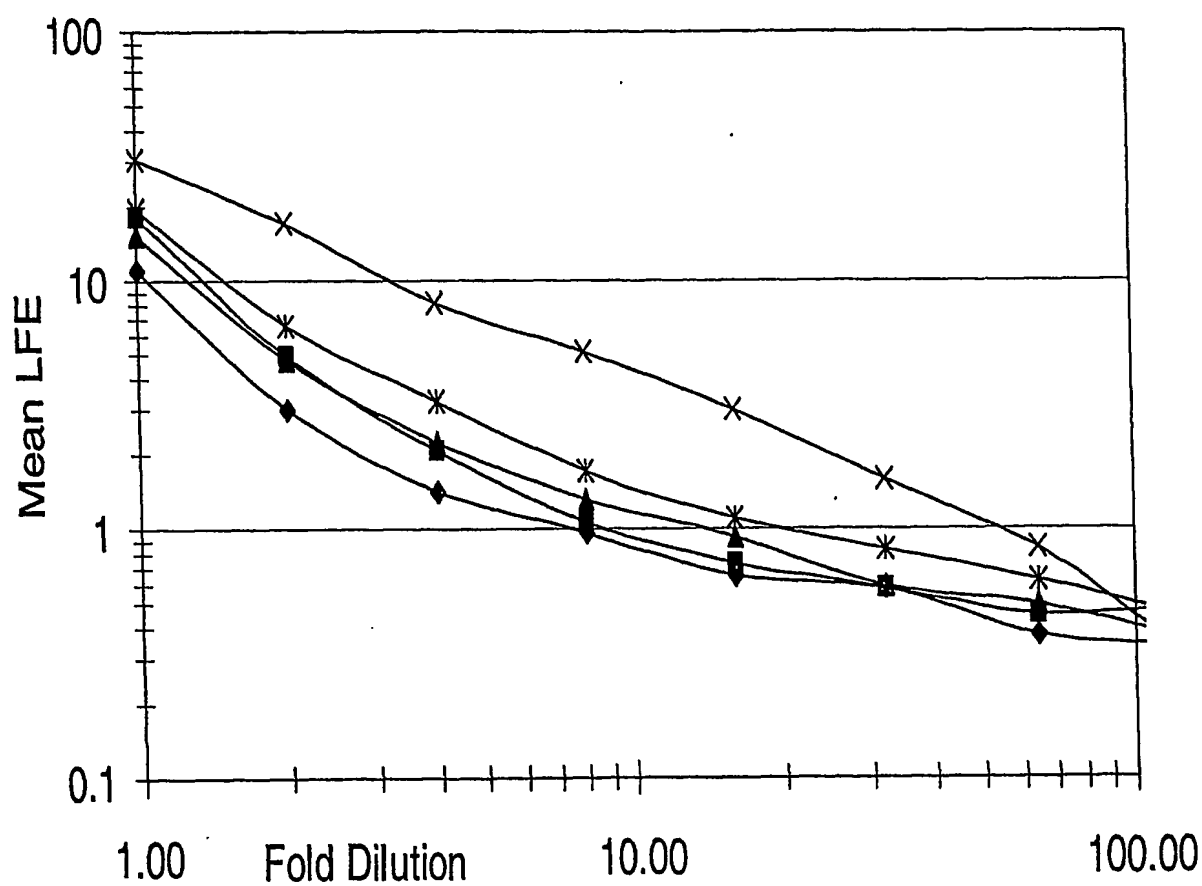


Fig. 35

Immunoblot Analysis of protein immunoprecipitates  
from COS transfections of 2H7 scFvIg Constructs

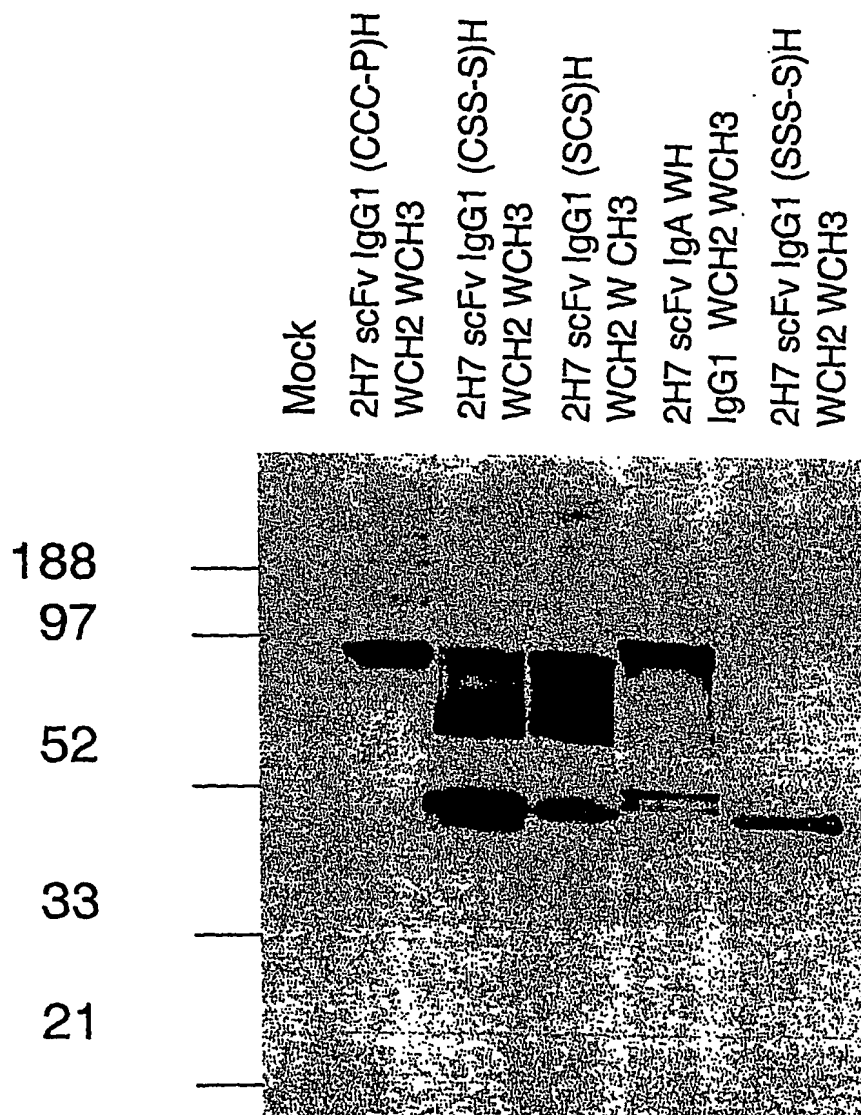


Fig. 36

Binding to CD20 CHO cells by constructs  
that link anti-CD20 scFv to IgA Fc Domains

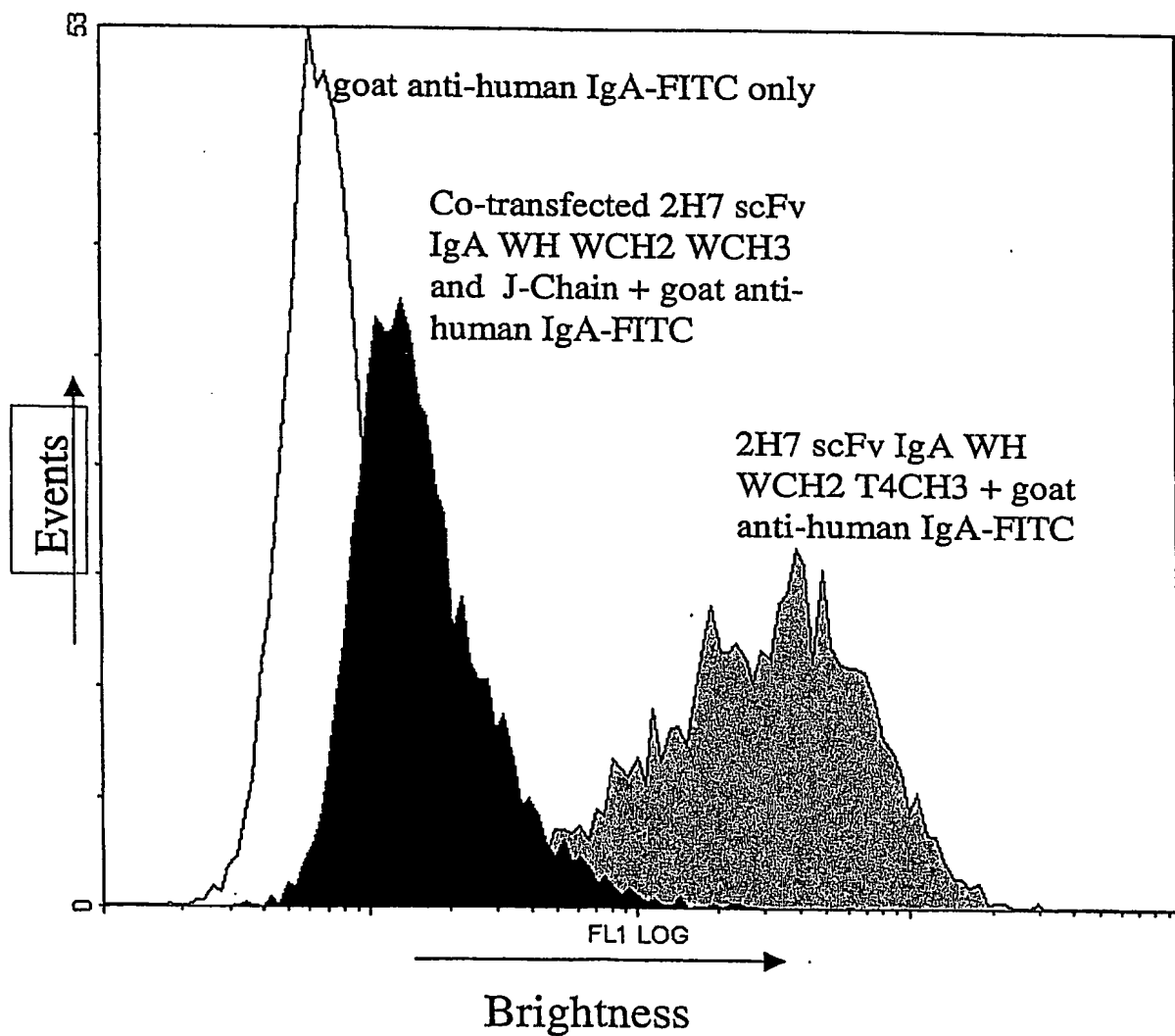




Fig. 37

Titration of CD20 specific scFvIg Constructs  
for ADCC Activity Using Whole Blood Effectors

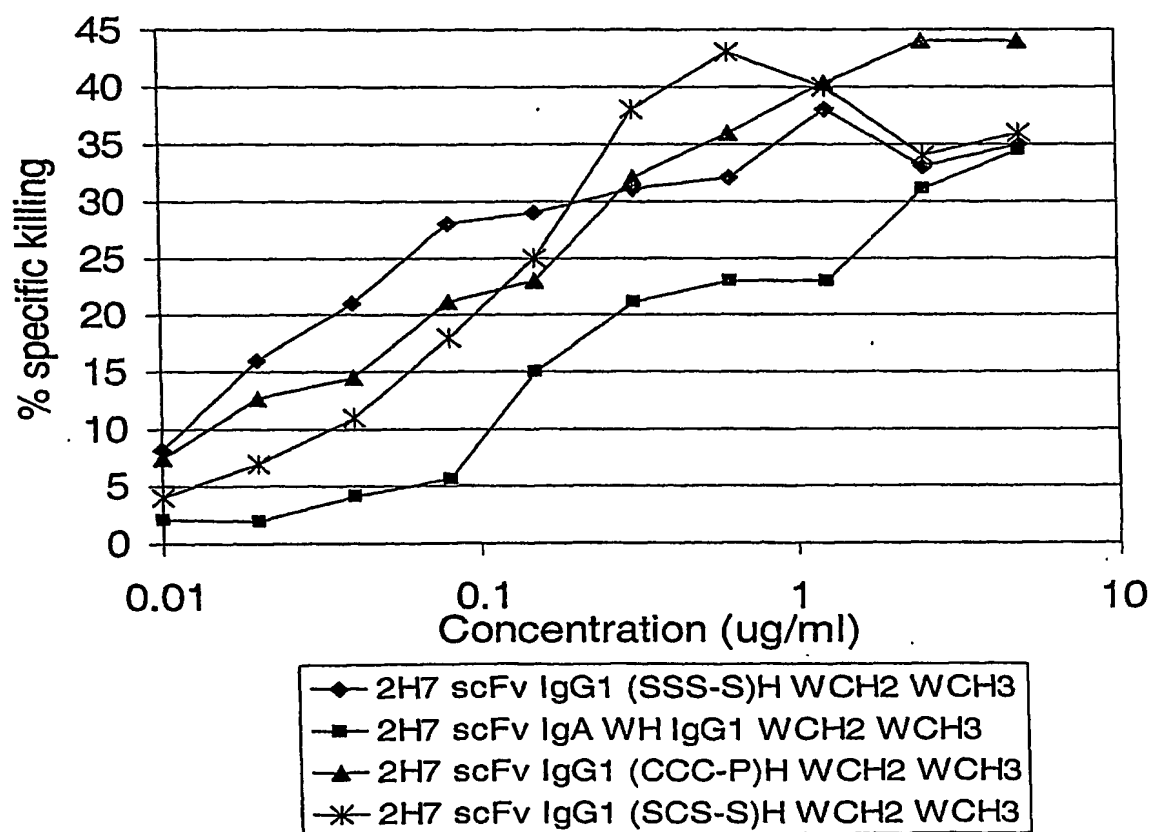


Fig. 38

ADCC Assay of anti-CD20 constructs with alternative tails  
(Whole Blood Effectors / BJAB Targets)

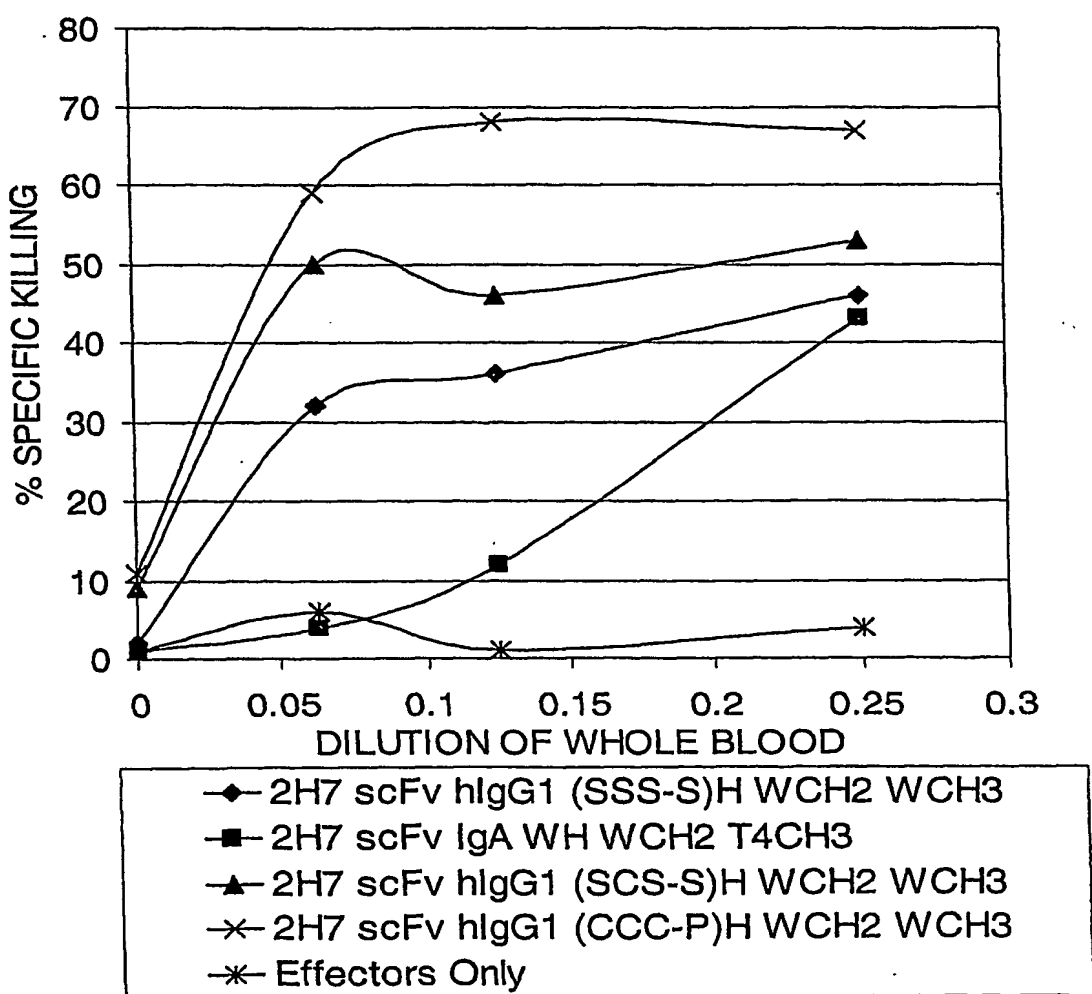


Fig. 39

# ADCC Assay of Anti-CD20 scFvIg Constructs Using Different Effector Populations Against BJAB Targets

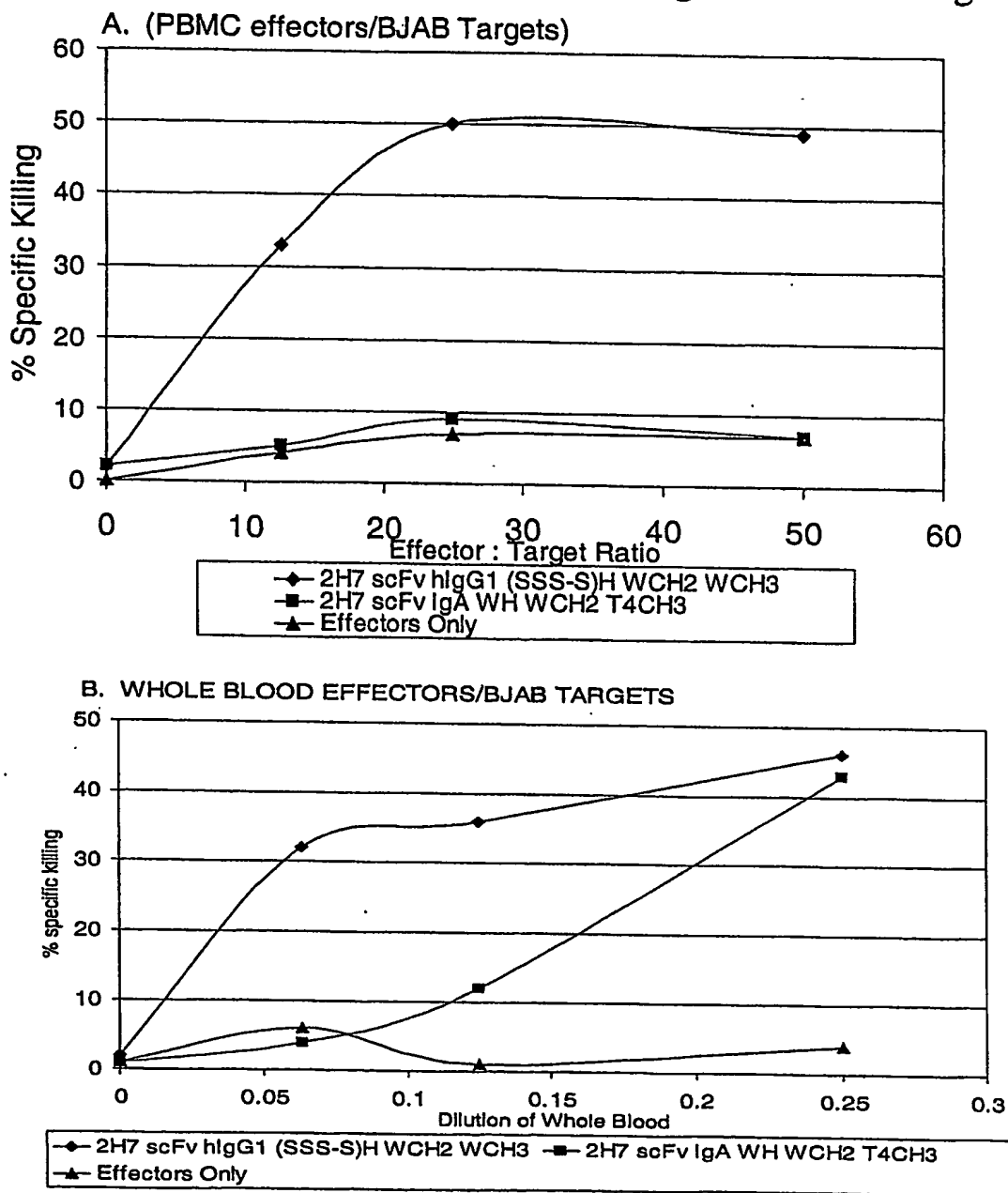
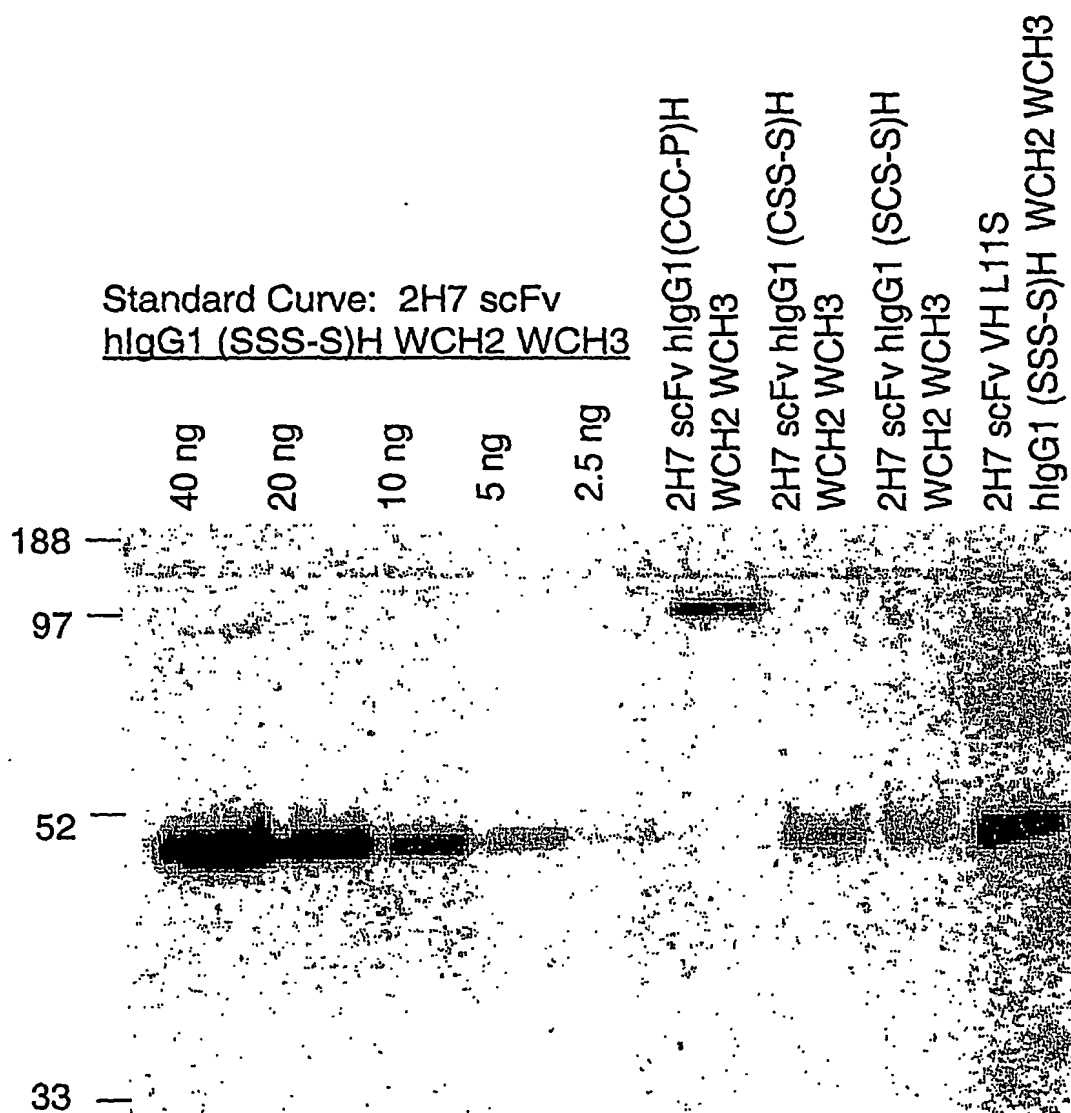


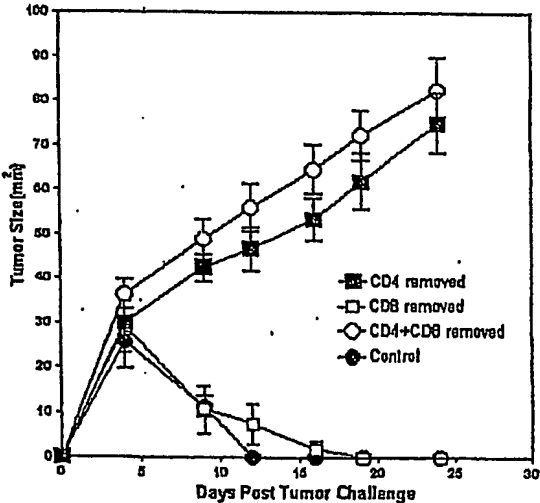
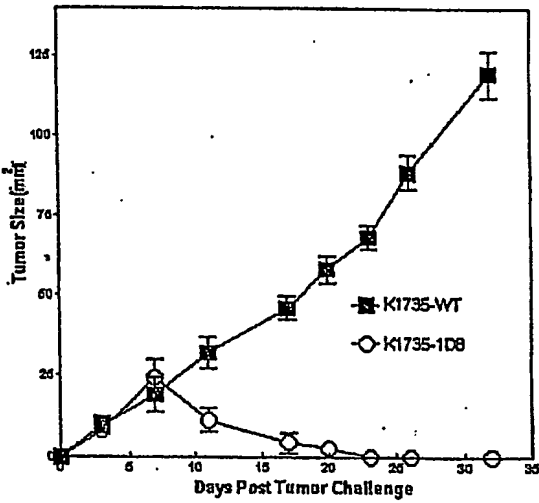
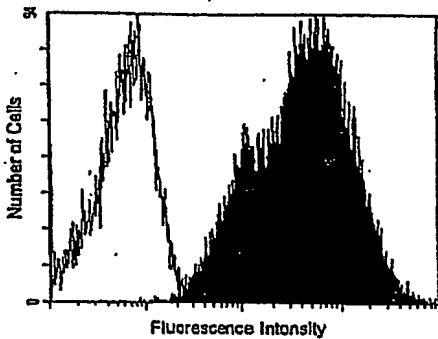
Fig. 40

Immunoblot of 2H7 scFv Ig constructs from COS  
Transfections (1  $\mu$ l/well) compared to a Concentration Standard



Figures 41A, 41B and 41C

A.



B.

C.

Fig. 42

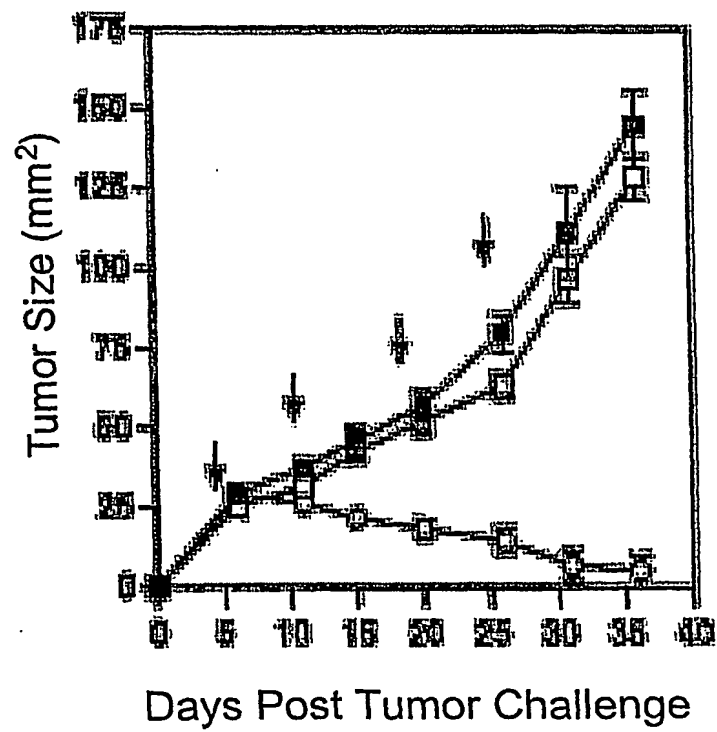


Fig. 43

Mixtures of K1735-WT and K1735-1D8 transfected tumor lines  
inhibit tumor outgrowth in C3H mice

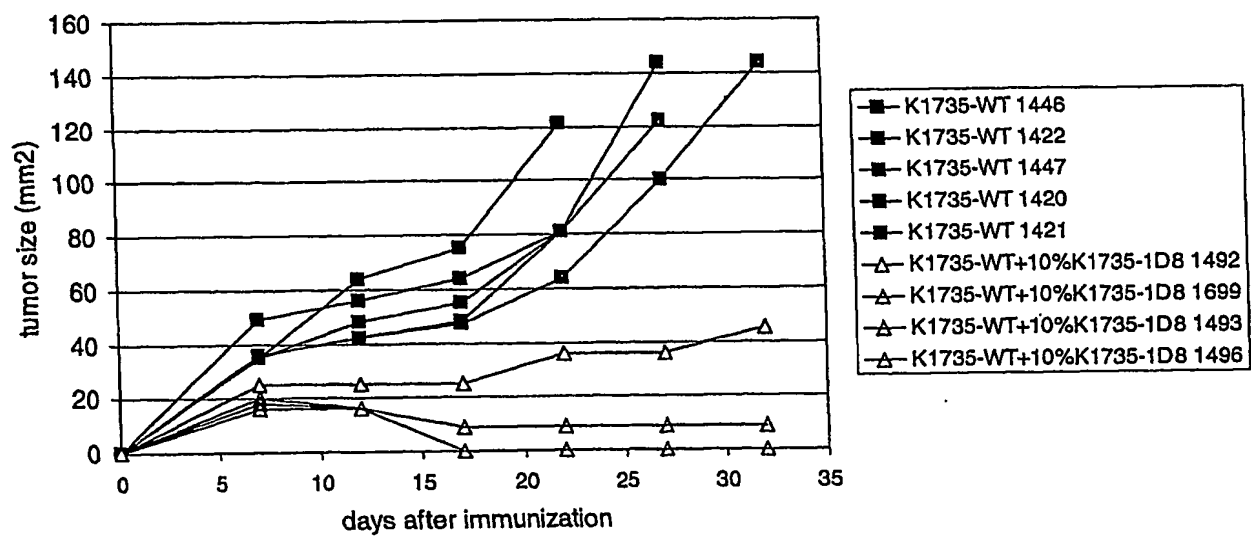


Fig. 44

Expression of anti-mouse CD137 (1D8) scFv-hIgG1 (SSS-S)H P238SCH2 WCH3  
On the surface of panned Ag104-1D8 Transfected Tumor Cells

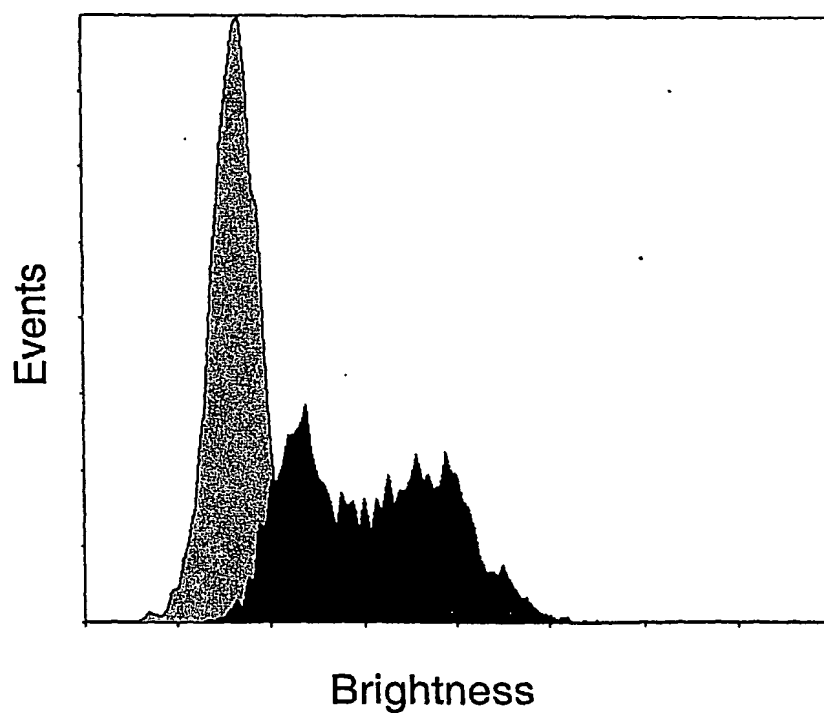




Fig. 45

Coomassie Stained SDS-PAGE Gel of 2H7 scFv Ig  
Constructs

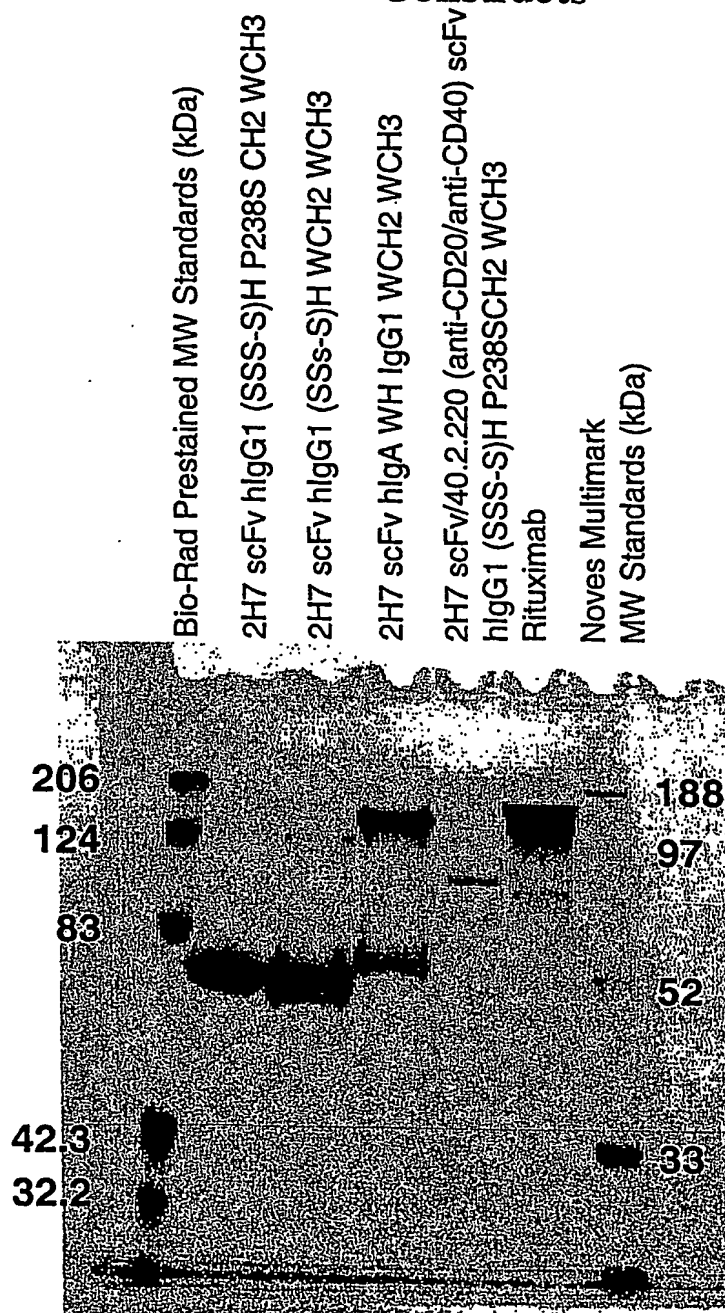
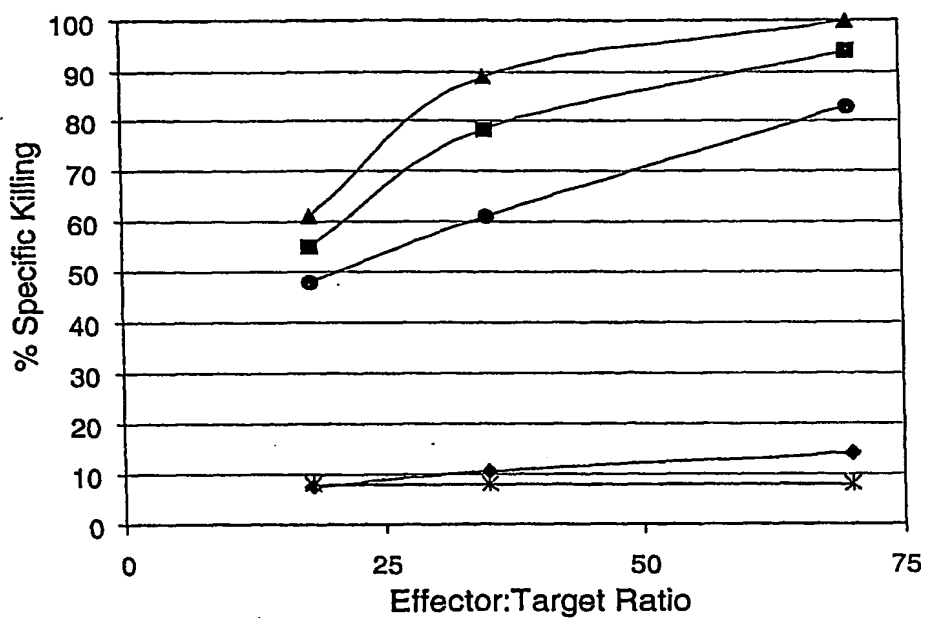


Fig. 46

ADCC mediated by 2H7 scFvIg Constructs by human  
PBMC effector cells against Bjab targets



◆ 2H7 scFv hIgG1(SSS-S)H P238SCH2 WCH3

▲ 2H7 scFv hIgA WH IgG1 WCH2 WCH3

■ 2H7 scFv hIgG1 (SSS-S)H WCH2 WCH3

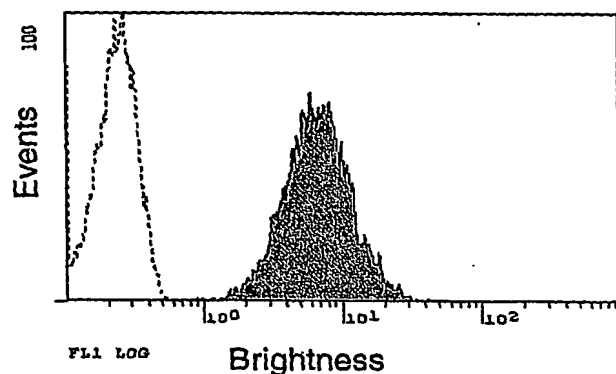
● RITUXIMAB

\* CELLS ALONE (W/O AB)

Fig. 47

Cell surface expression of anti-human CD3 G19-4  
scFv hIgG1 (SSS-S)H P238SCH2 WCH3-  
hCD80TM/CT on Reh and T51 Cells.

Reh anti-CD3 (G19-4) scFv hIgG1 (SSS-S)H  
P238SCH2 WCH3-hCD80TM/CT



T51 G19-4 scFv hIgG1 (SSS-S)H  
P238SCH2 WCH3-hCD80TM/CT:

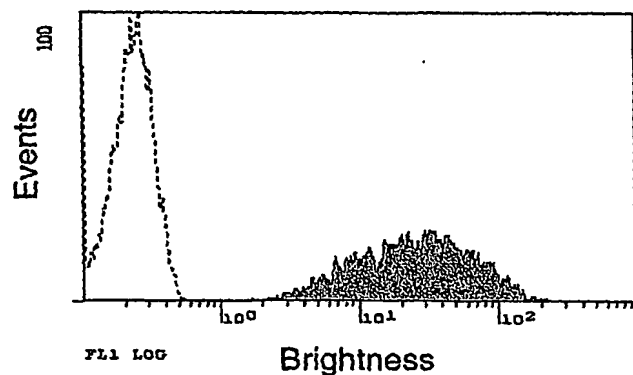
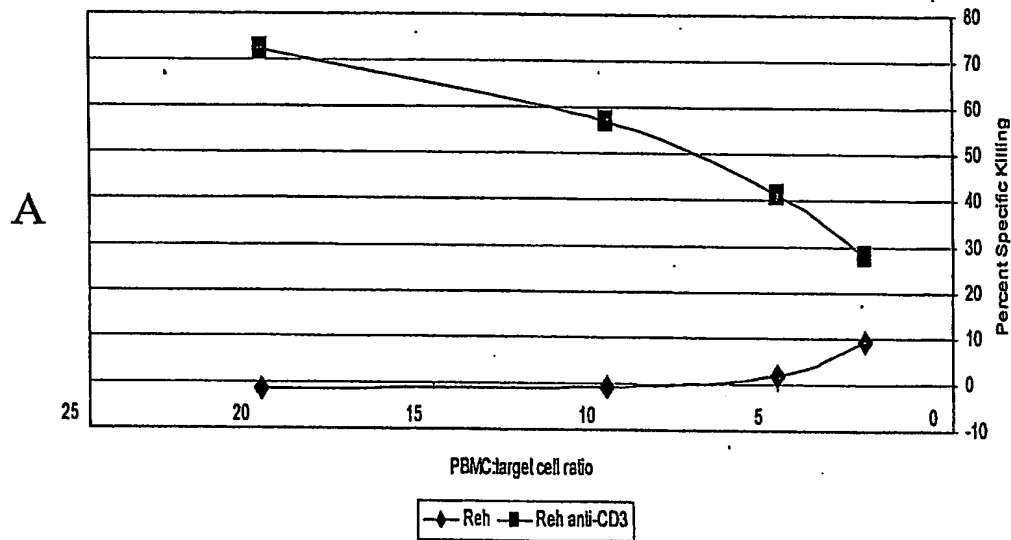


Figure 48.

### Targeting of Cytotoxicity to Transfected Cell Lines by Surface expression of CD3 scFvIg

Cytotoxic activity of resting PBMC towards transfected Reh cells



Cytotoxic activity of resting PBMC towards transfected T51 lymphoblastoid cells

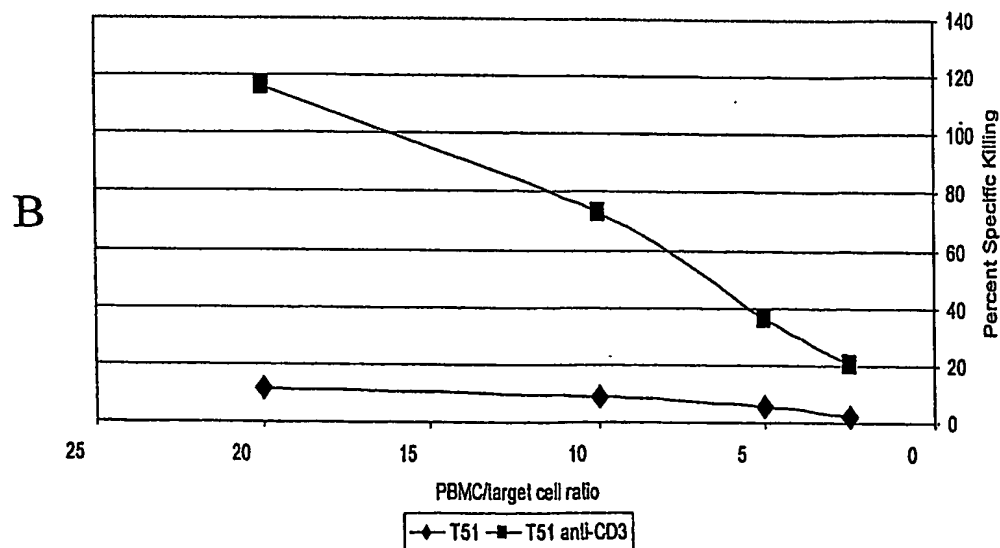


Fig. 49

Binding of 5B9, a mouse anti-human CD137 scFv hIgG1  
(SSS-S)H WCH2WCH3 to stimulated human PBMC

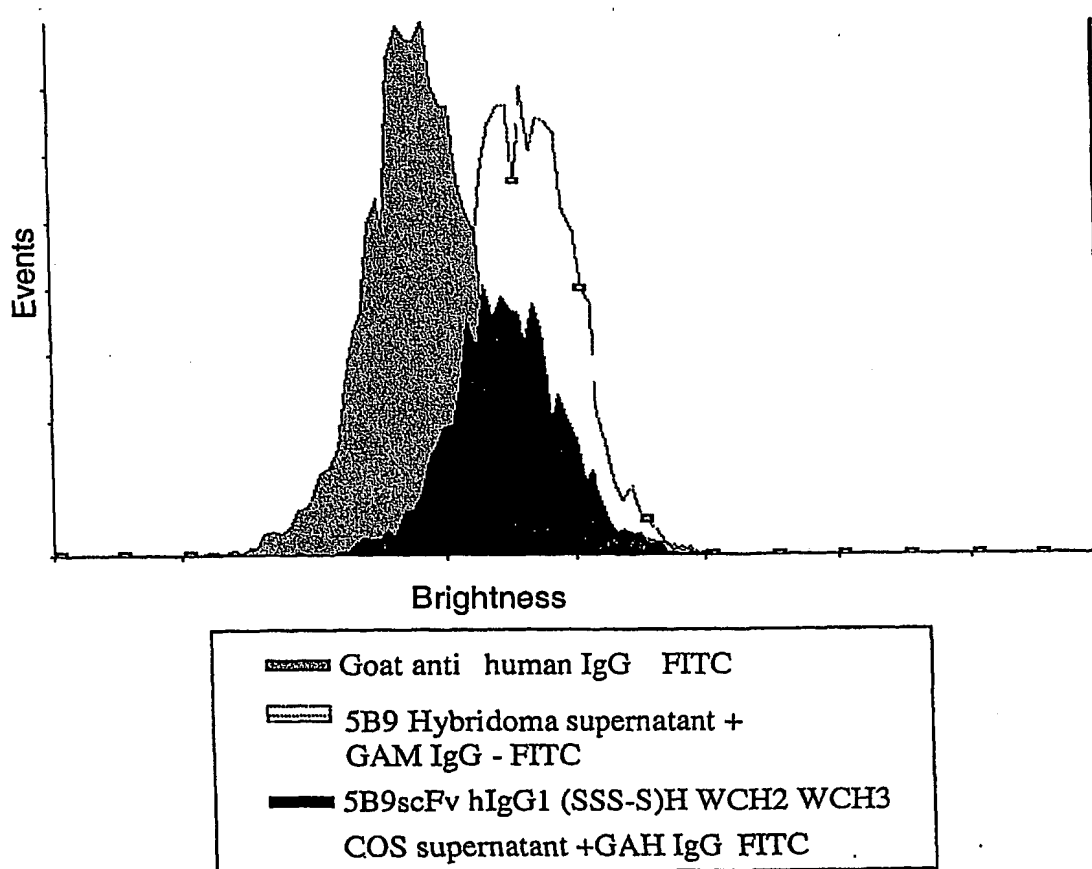
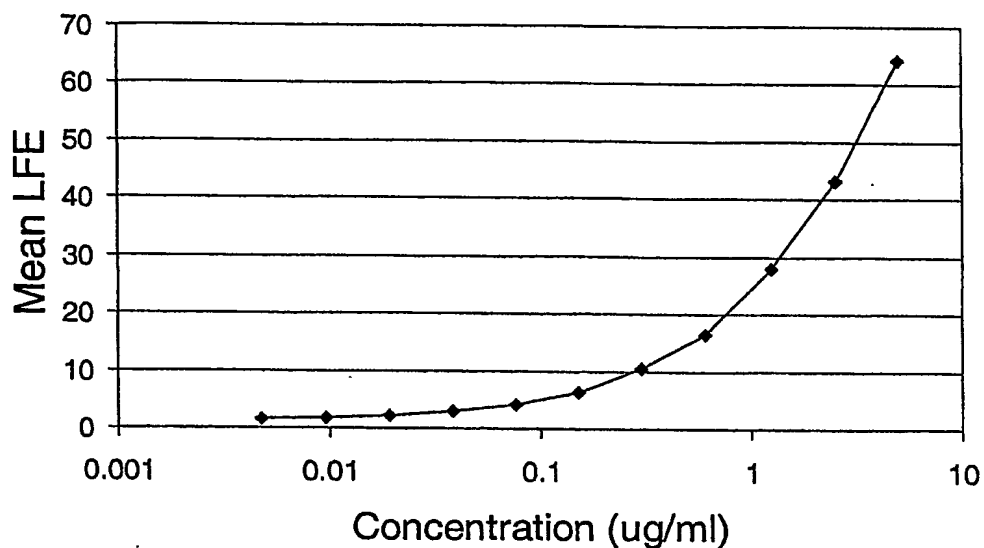


Fig. 50

Effect of V<sub>H</sub>L11S Mutation on Cytox B20  
 2H7 scFv hIgG1 (SSS-S)H WCH2 WCH3 Protein Expression

50A. Standard Curve: 2H7VH-L11S-IgG1 (SSS-S)H WCH2 WCH3



50B. CHO supernatant Brightness and Estimation of Protein concentrations from Standard Curve:

|                          | CHO clone name |      |      |       |       |
|--------------------------|----------------|------|------|-------|-------|
|                          | 4F2            | 4F5  | 3E5  | 6B11A | 2B8A  |
| Mean LFE                 |                |      |      |       |       |
| 1/100                    | 71.7           | 40.6 | 31.5 | 99.7  | 101.5 |
| 1/500                    | 27.1           | 12.4 | 11.2 | 40.8  | 43    |
| approx<br>conc.<br>μg/ml | 600            | 225  | 125  | 1000  | 1250  |

Fig. 51

Production Levels of 2H7scFv VH L11S hIgG1  
(SSS-S)H WCH2 WCH3  
From CHO Clone Culture Supernatants

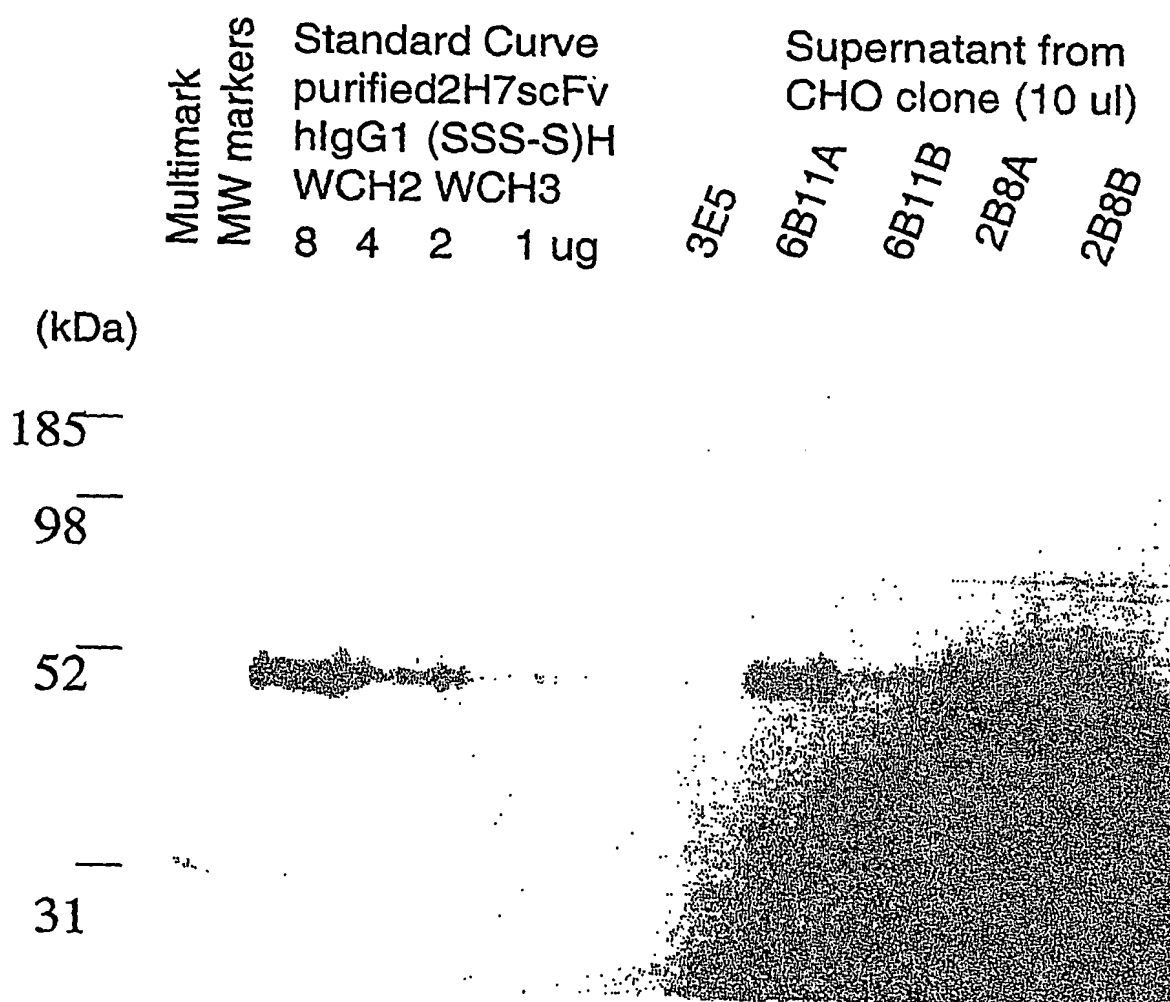
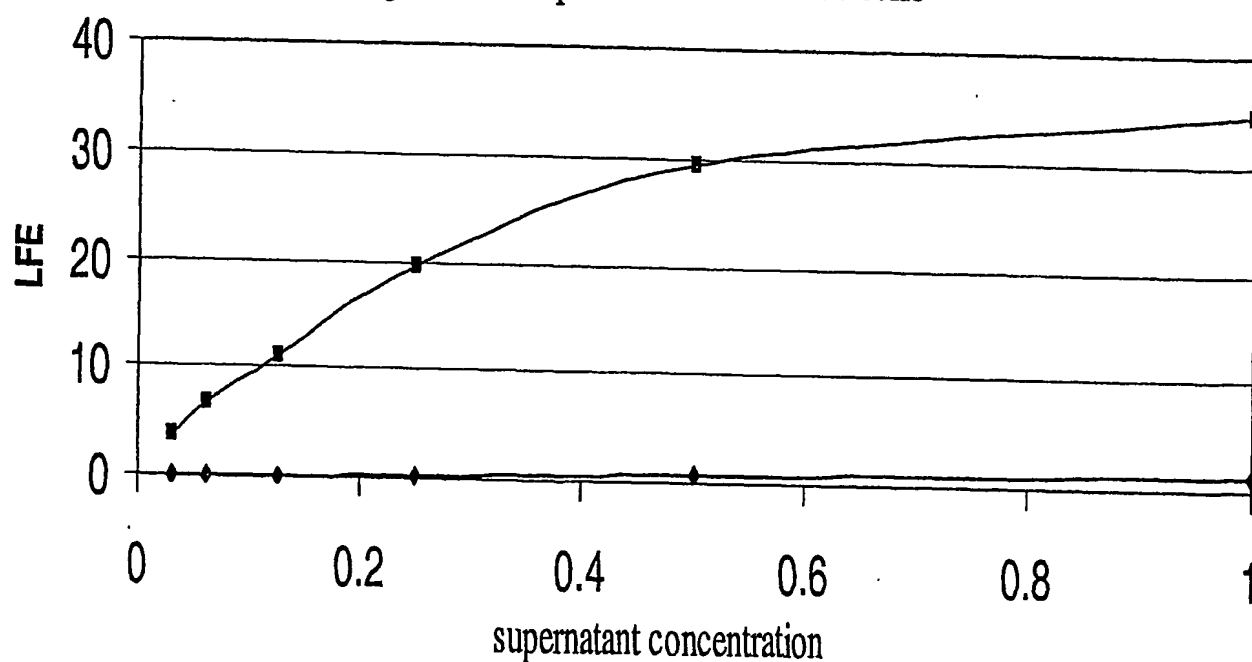


Fig. 52

# Effect of VHL11S Mutation on G28-1 scFvIg Construct Protein Production from COS cells

Binding of COS supernatants to Ramos cells



◆ G28.1 scFv hIgG1(SSS-S)H WCH2 WCH3    ■ G28.1 scFv VHL11S hIgG1(SSS-S)H WCH2 WCH3



# Fig. 53

## Immunoblot of G28-1 scFvIg Constructs

Increased Protein Levels in COS supernatants  
transfected with G28-1scFv hlgG1 (SSS-S)H WCH2 WCH3  
After Substitution of Leucine with Serine at position 11 of VH (VHL11S)

Fig. 53A.

|                             |      |      |      |                              |   |   |   |   |
|-----------------------------|------|------|------|------------------------------|---|---|---|---|
| Purified G28-1<br>(11/6/01) |      |      |      | G28-1 scFv<br>hlgG1 (SSS-S)H |   |   |   |   |
| scFv IgG1 (SSS-S)H          |      |      |      | WCH2 WCH3                    |   |   |   |   |
| WCH2 WCH3                   |      |      |      | 1 ul/well                    |   |   |   |   |
| 80ng                        | 40ng | 20ng | 10ng | A                            | B | C | D | E |

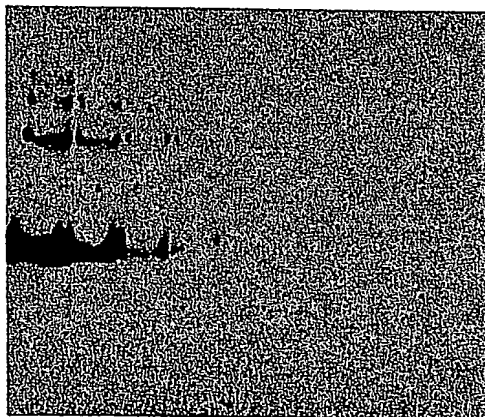


Fig. 53B.

|                             |      |      |      |                                    |   |   |   |   |
|-----------------------------|------|------|------|------------------------------------|---|---|---|---|
| Purified G28-1<br>(11/6/01) |      |      |      | G28-1VHL11S<br>scFv hlgG1 (SSS-S)H |   |   |   |   |
| scFv hlgG1 (SSS-S)H         |      |      |      | WCH2 WCH3                          |   |   |   |   |
| WCH2 WCH3                   |      |      |      | 1 ul/well                          |   |   |   |   |
| 80ng                        | 40ng | 20ng | 10ng | A                                  | B | C | D | E |

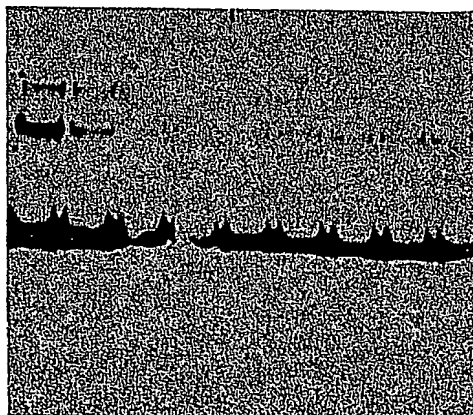


Fig. 54

Binding of 2H7 scFvIg Constructs with Altered Hinges and CH3 domains to CD20 CHO Cells

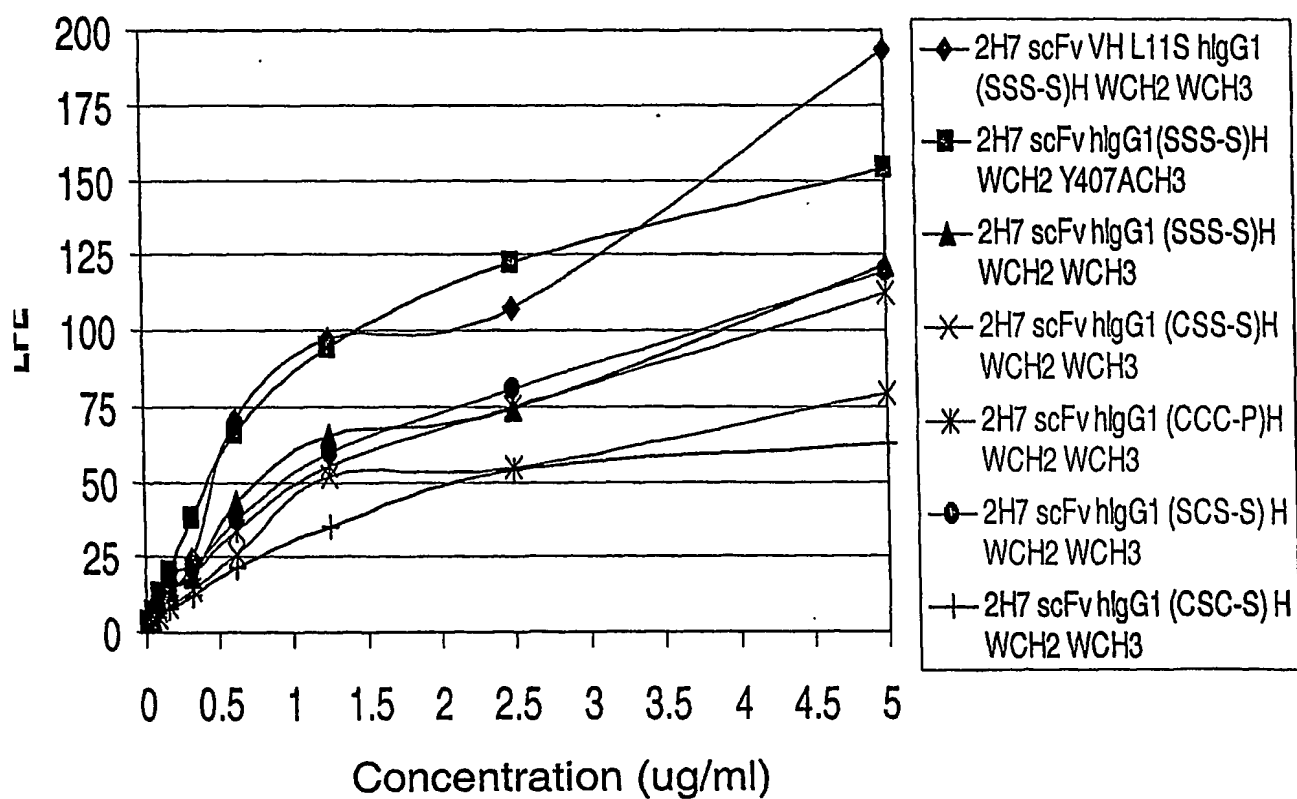


Fig. 55

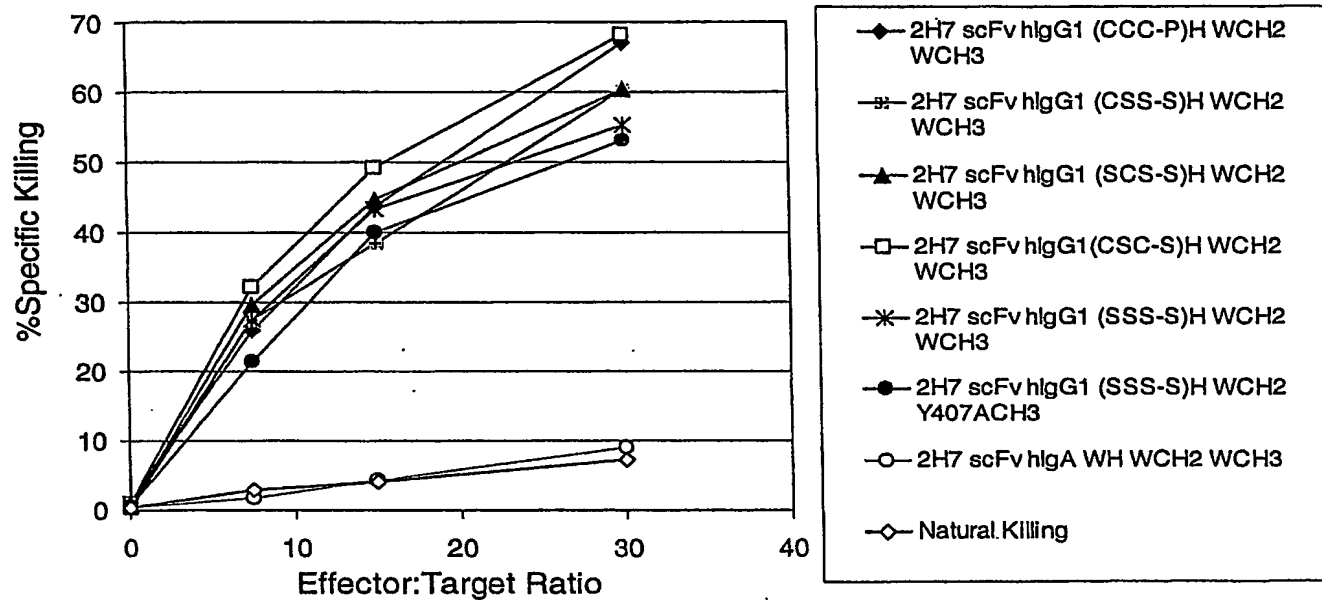
ADCC Activity of 2H7 scFvlg constructs Against  
BJAB Targets and PBMC Effectors

Fig. 56

### Complement Activity of 2H7 scFvlg Constructs With Ramos Target Cells

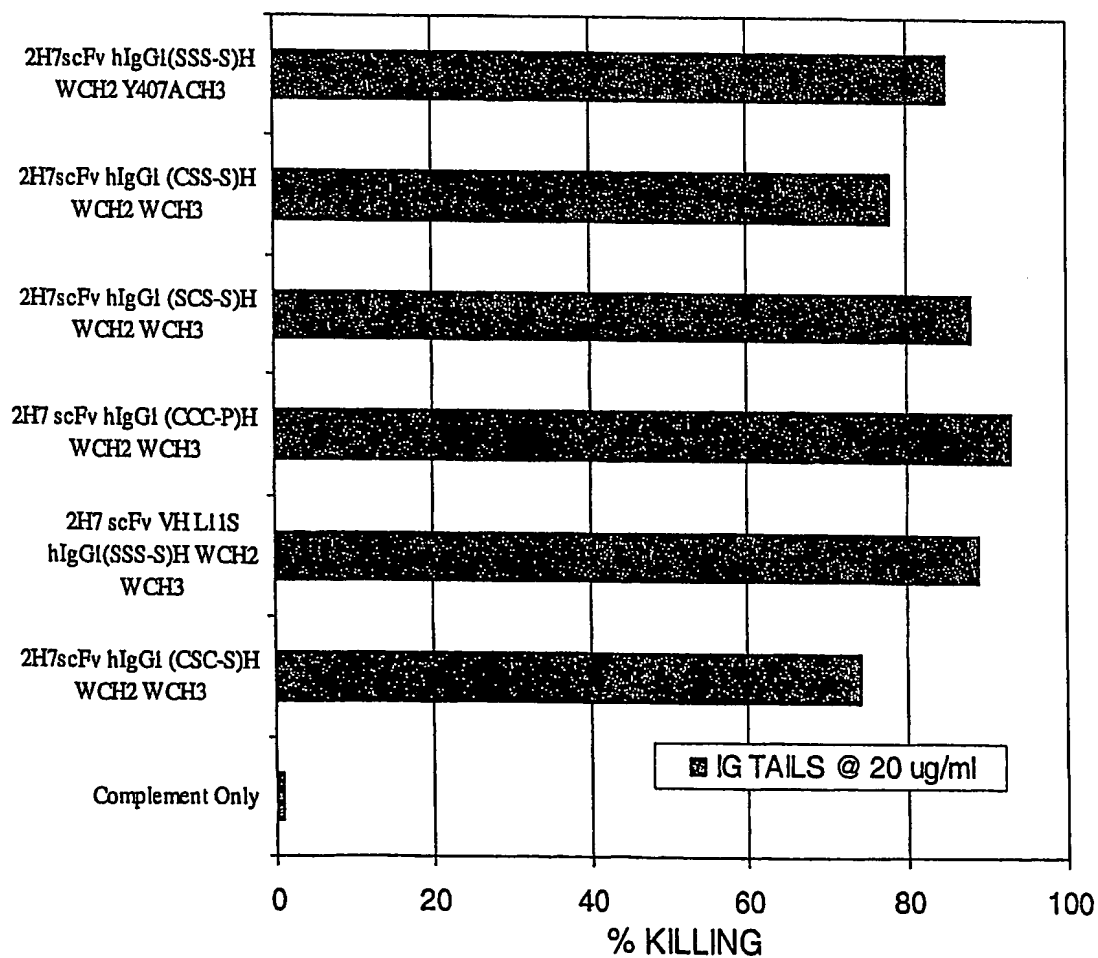
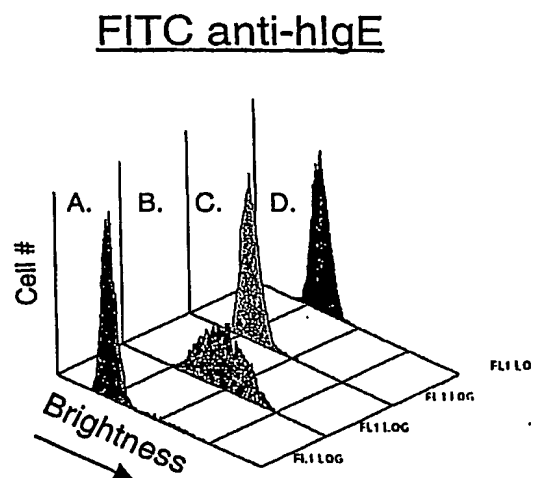
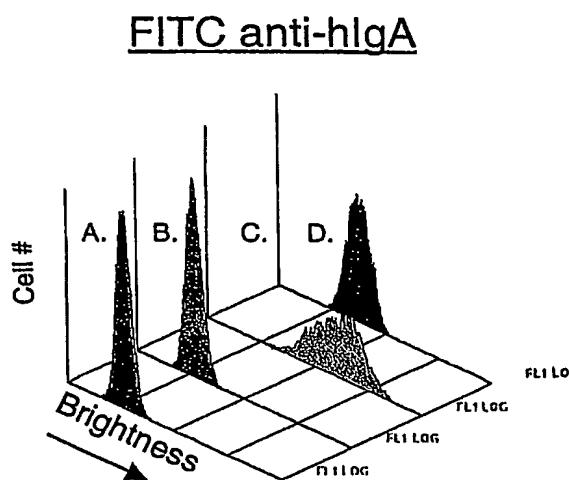
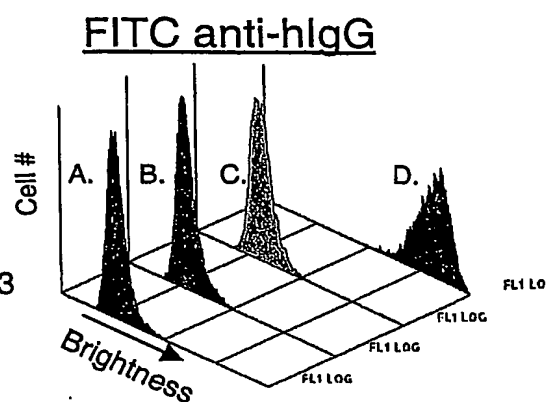


Fig. 57

## Binding of 2H7 scFvIg Derivatives to CD20CHO Cells

- A. ■ No fusion protein  
 B. ■ 2H7 scFv hlgE CH2CH3CH4  
 C. ■ 2H7 scFv hlgA WH WCH2 WCH3  
 D. ■ 2H7 scFv hlgG1 (SSS-S)H WCH2 WCH3



# Fig. 58

Fig. 58A. 2H7 scFv VHL11S human IgE (WCH2 WCH3 WCH4)  
Binding to CD20 CHO at 30 ug/ml

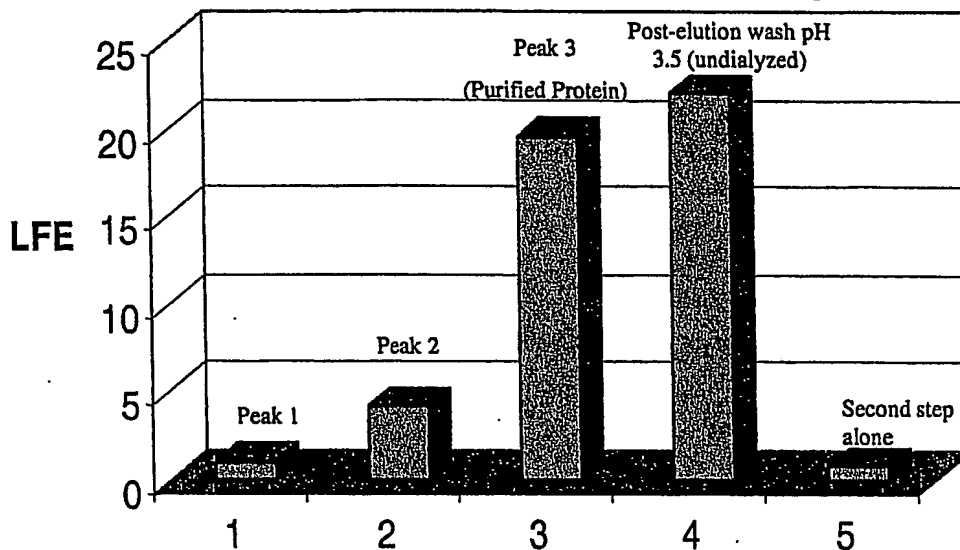


Fig. 58B. ADCC Activity of 2H7 VHL11S IgE (WCH2 WCH3 WCH4)  
Protein Fractions with PBMC Effectors and Bjab Targets

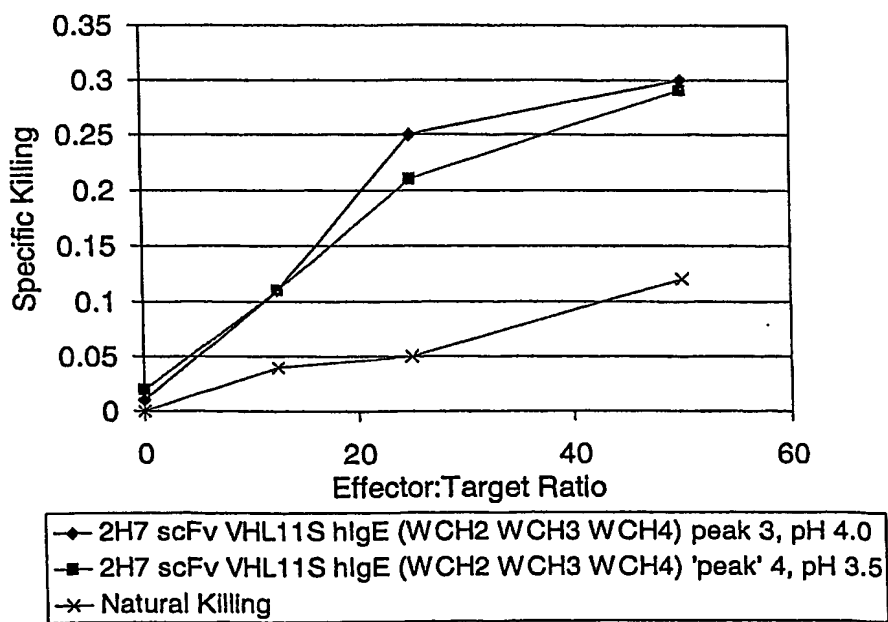


Fig. 59

Binding Data for COS derived  $\alpha$ -CD20 (2H7) scFv VHL11S  
mIg E (WCH2 WCH3 WCH4) and  
mIgA (WH WCH2 WCH3) Tailed Molecules

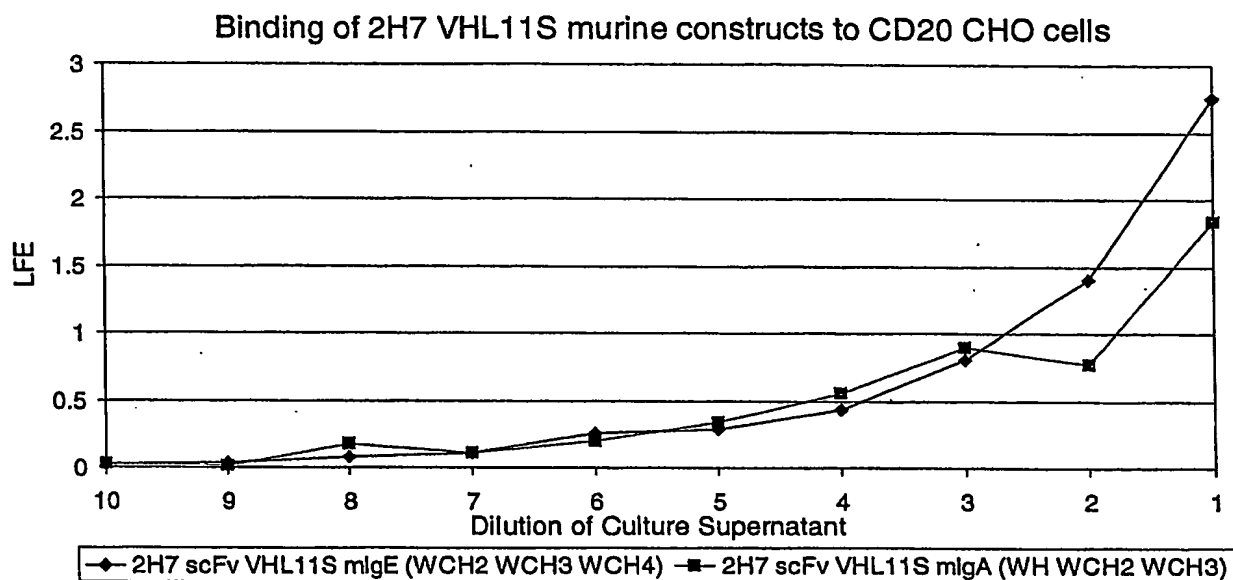


Fig. 60

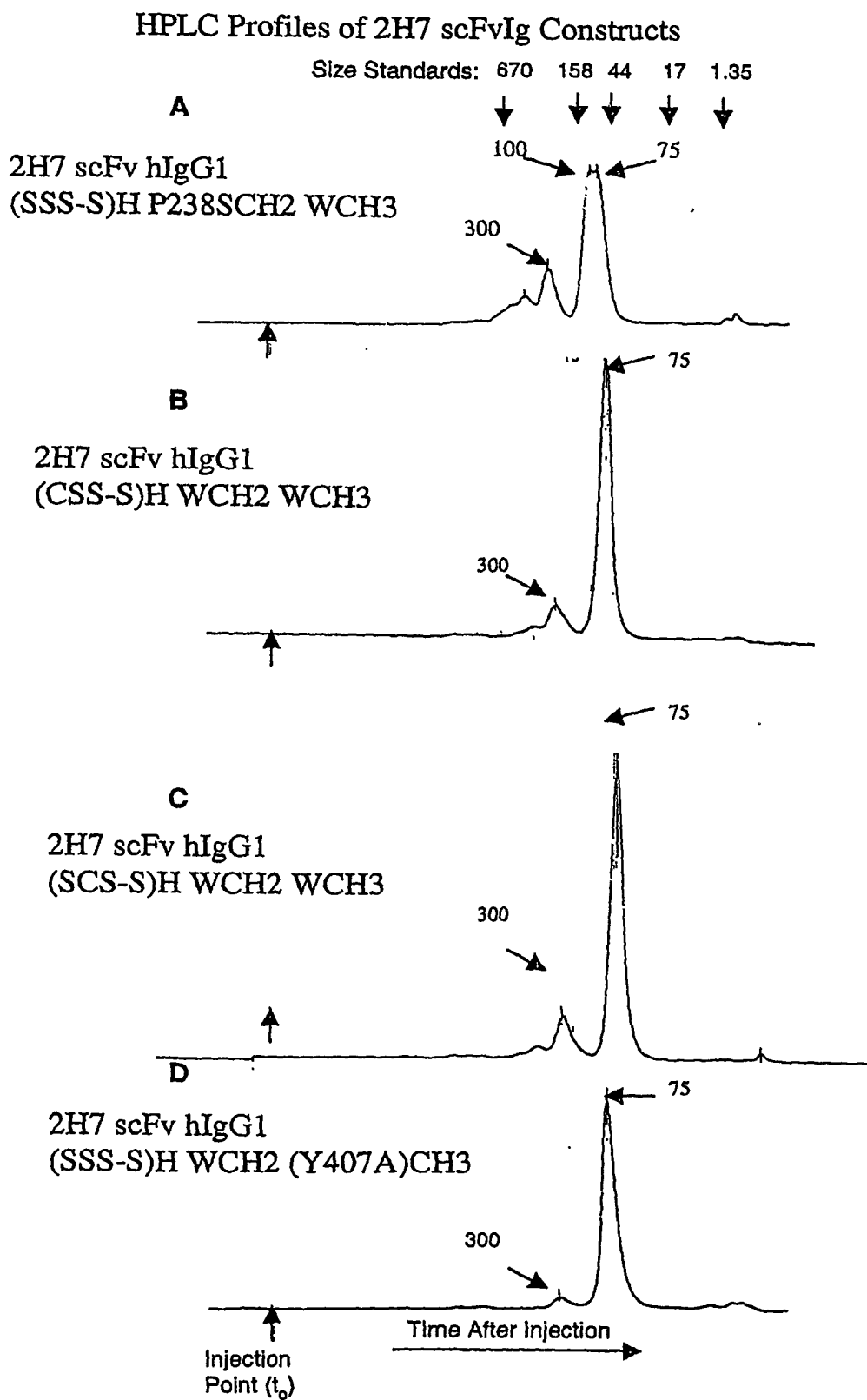




Fig. 61

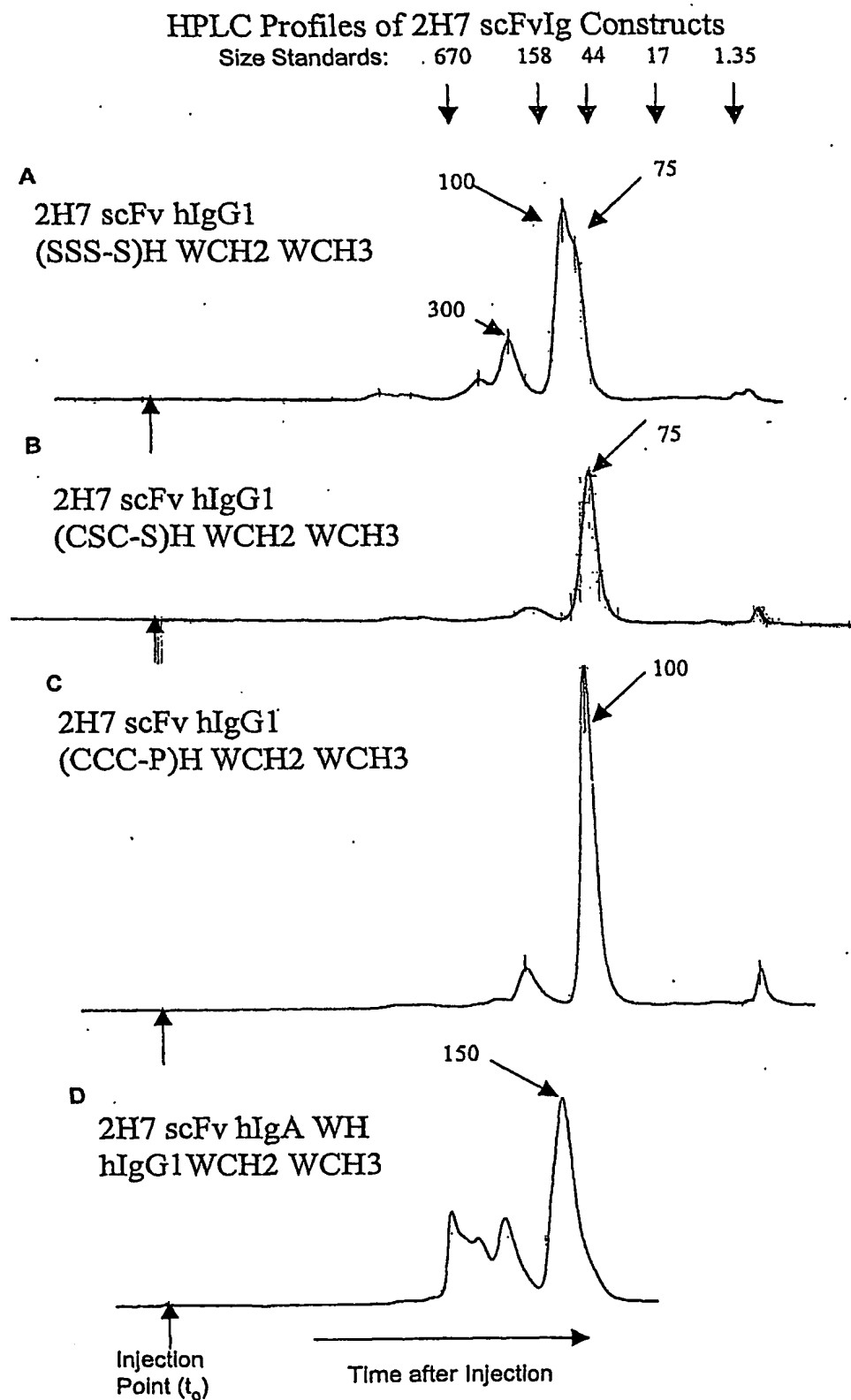


Fig. 62

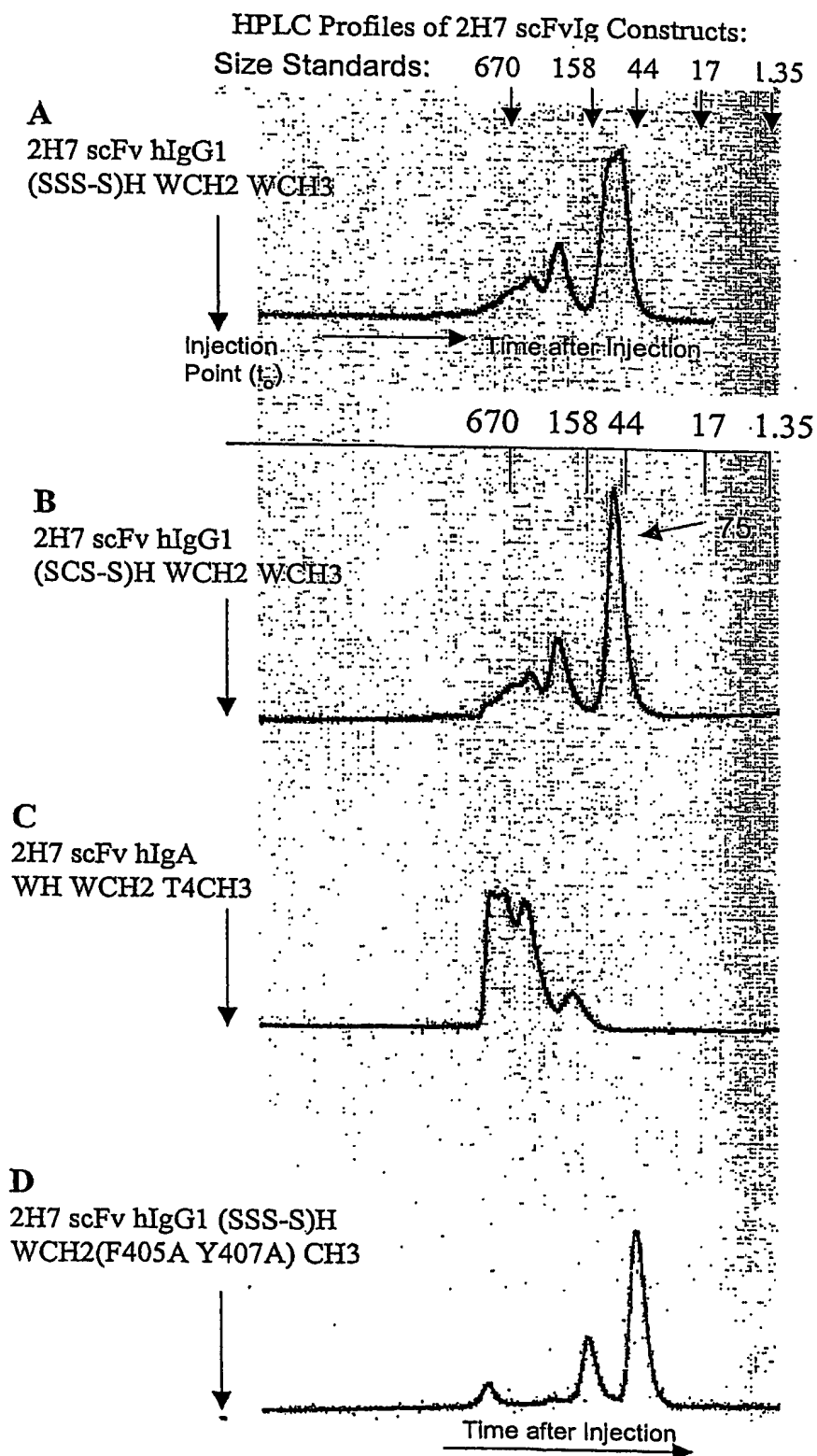


Fig. 63

Binding of Purified Proteins from COS Supernatants  
to CD20 CHO cells:  
Differential Effects of CH3 Mutations on Binding

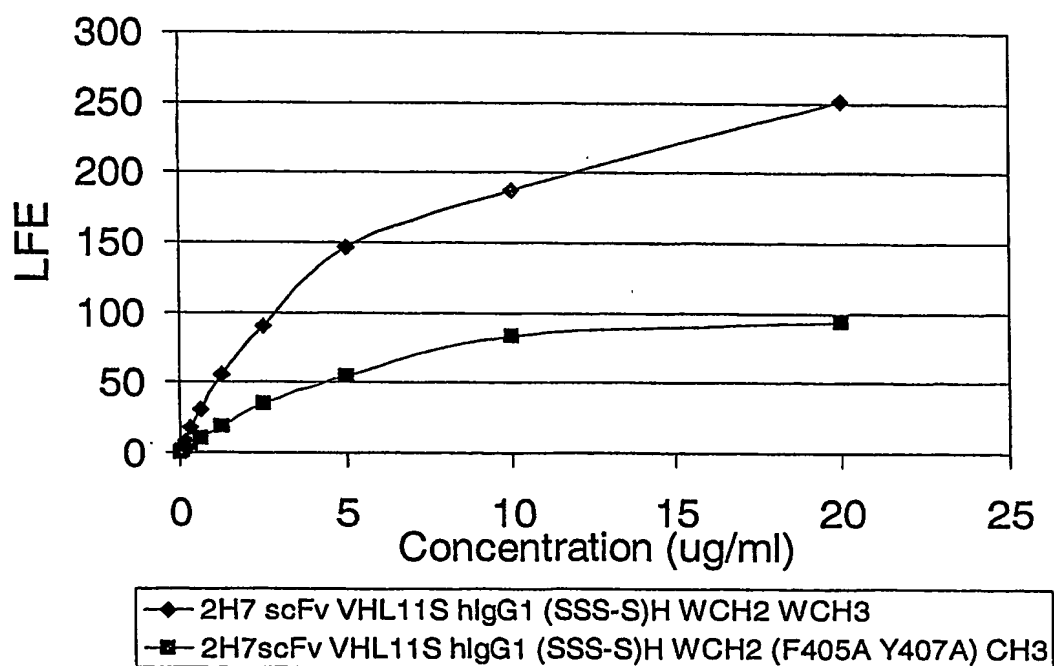


Fig. 64

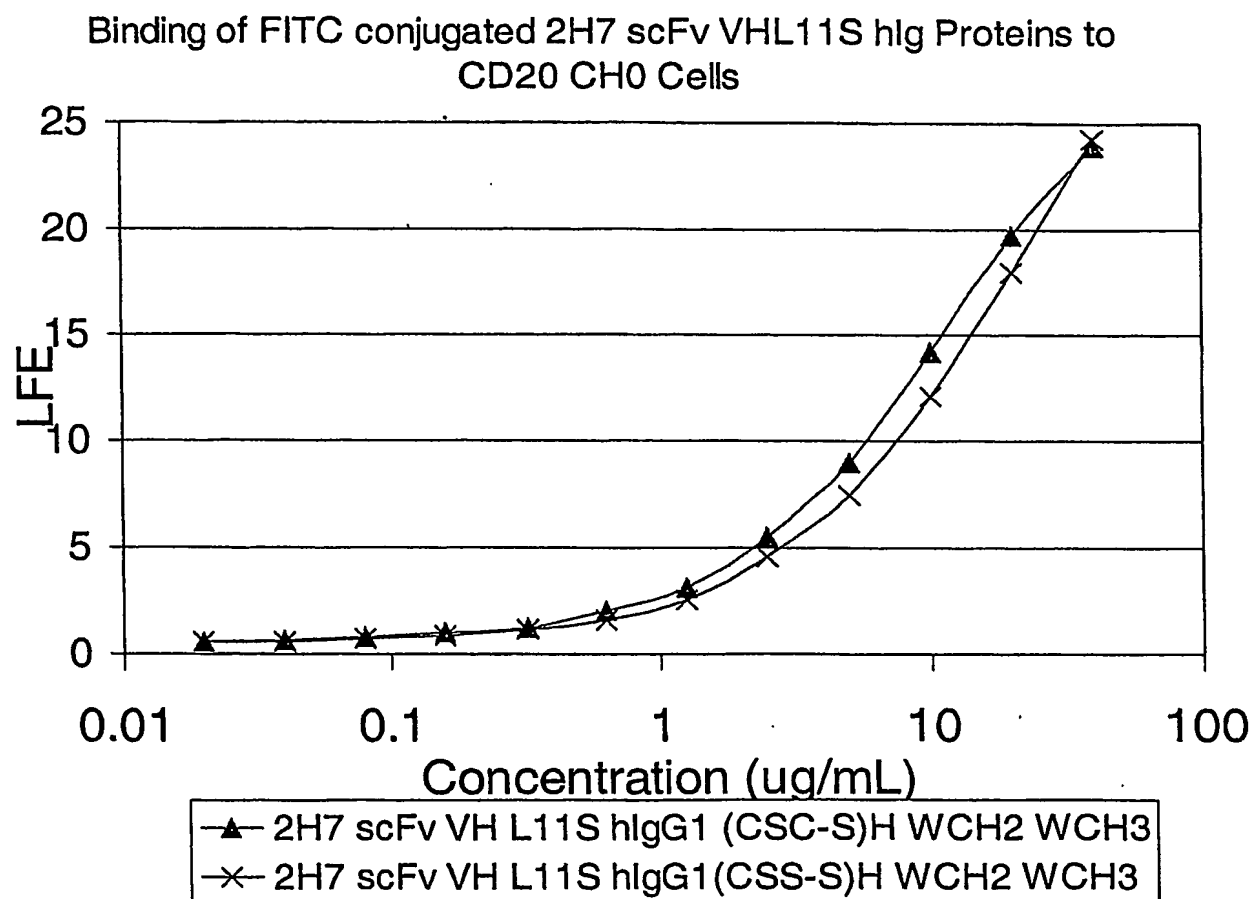


Fig. 65

Nonreducing SDS-PAGE on Protein A-Purified Lots  
of 2H7 scFv VHL11S hlg Constructs (10 ug/lane)

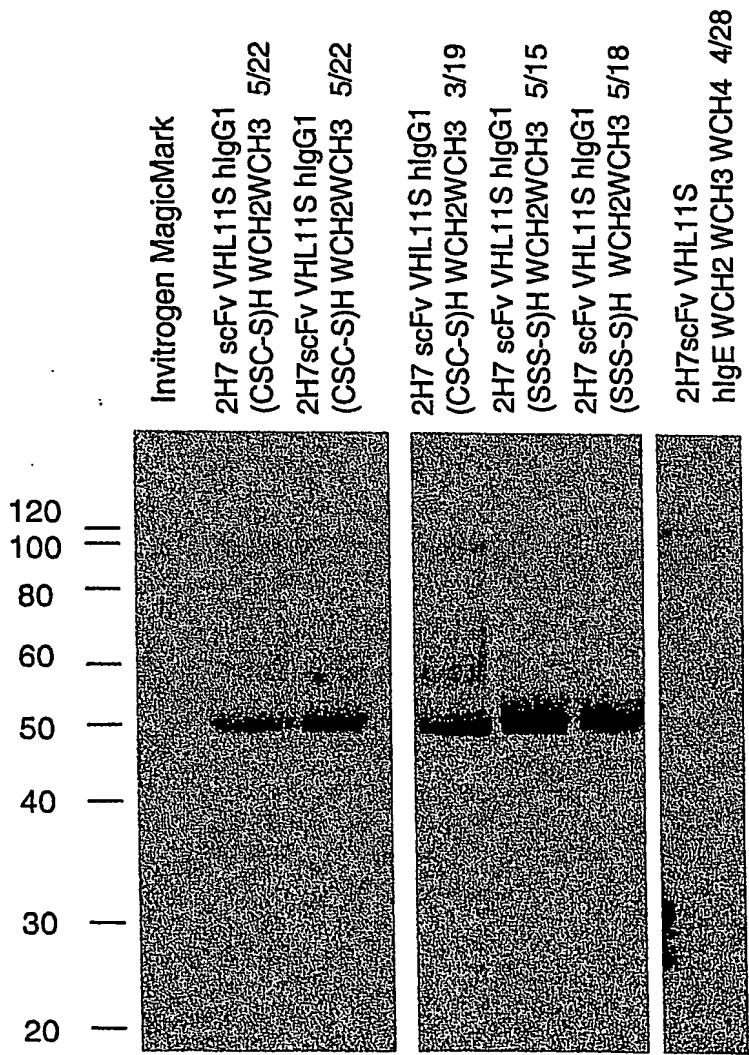


Fig. 66

# Alterations in Human IgG Fc sequence that differentially change effector function efficiency

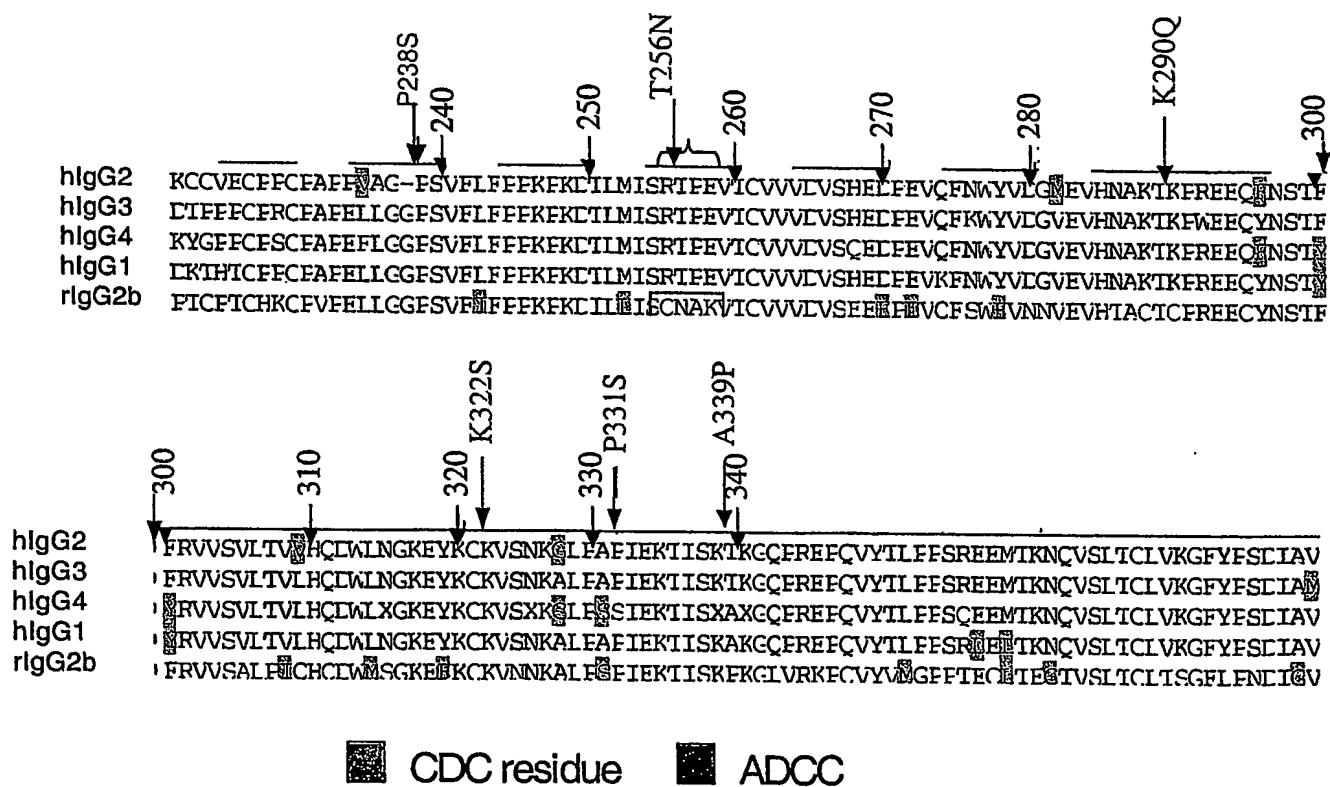


Figure 67.

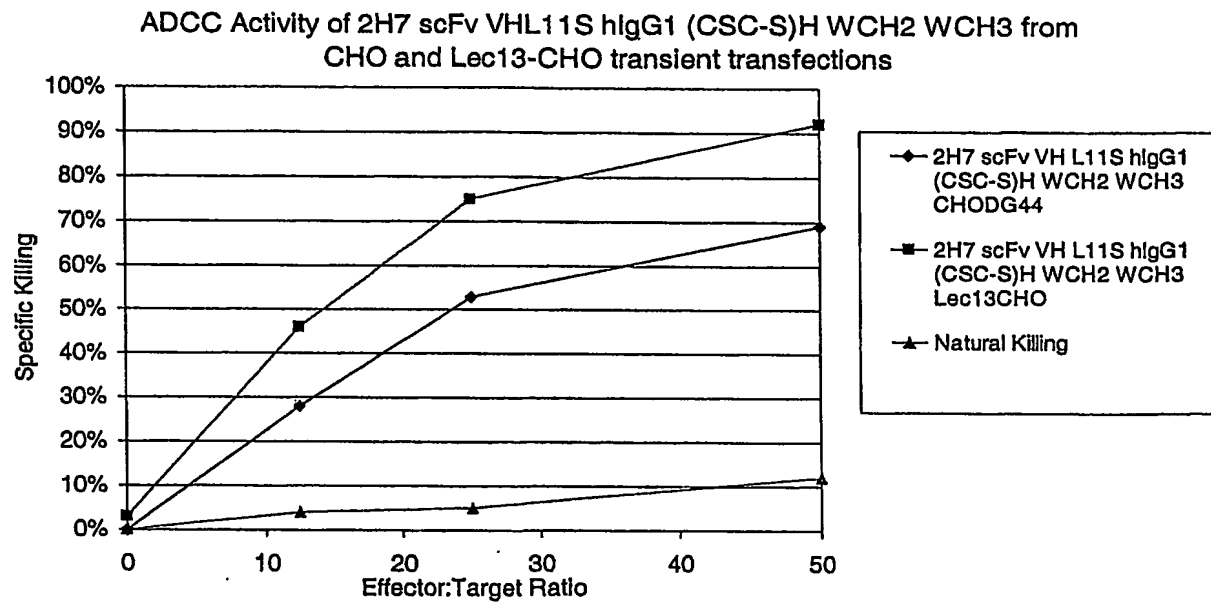


Fig. 68

CD16(ED) hIgG1(SSS-S)H P238S CH2 WCH3 high and low affinity alleles expressed as soluble molecules

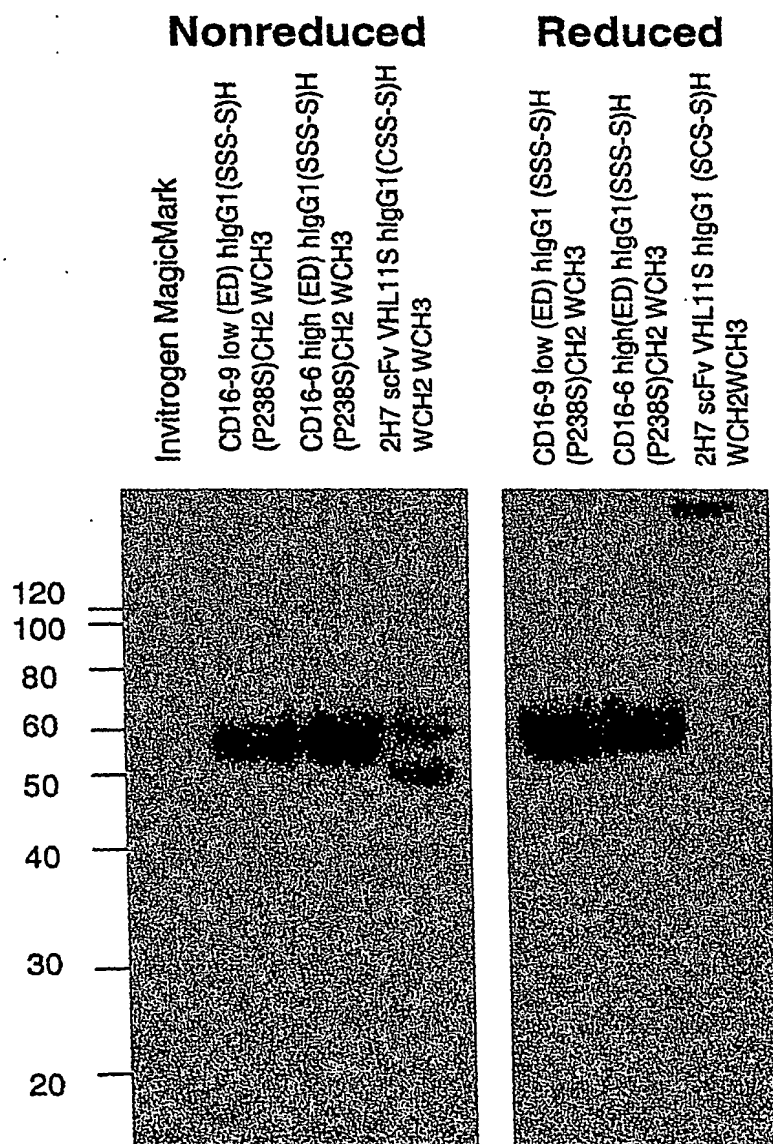




Fig. 69

Binding of soluble CD16-FITC high and low affinity fusion proteins to 2H7 scFv VHL11S hIgG1 (CSC-S)H WCH2WCH3 or (SSS-S)H (P238S)CH2WCH3 on CD20CHO Targets

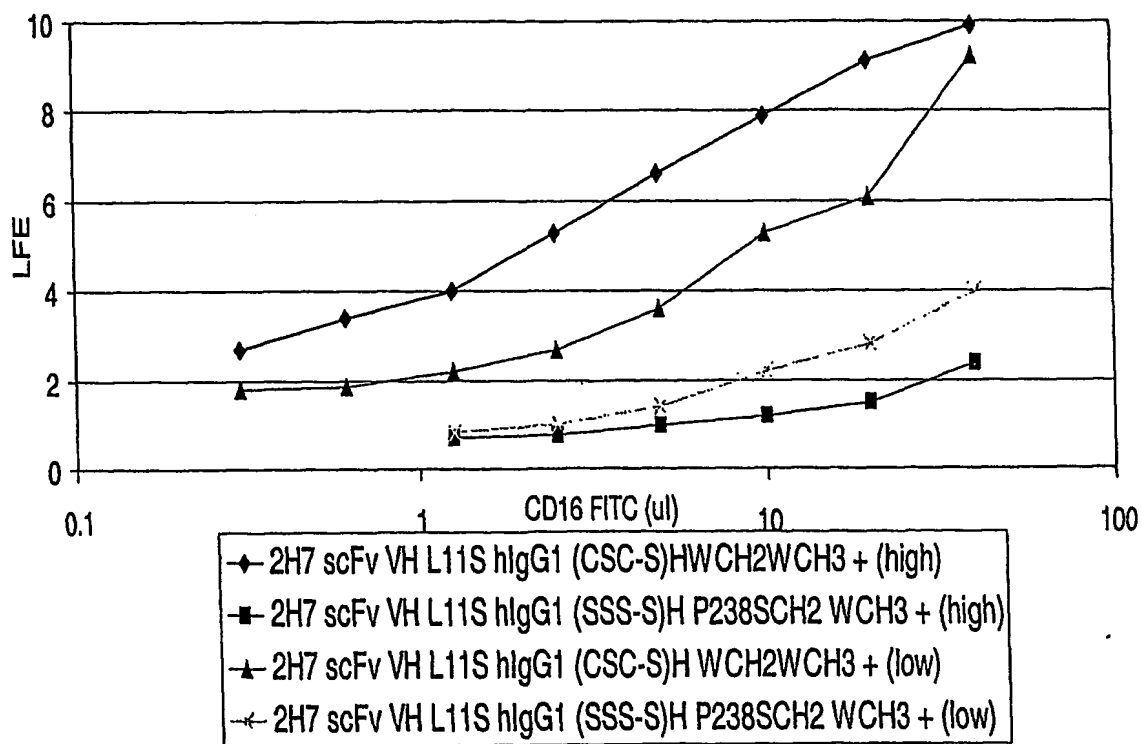
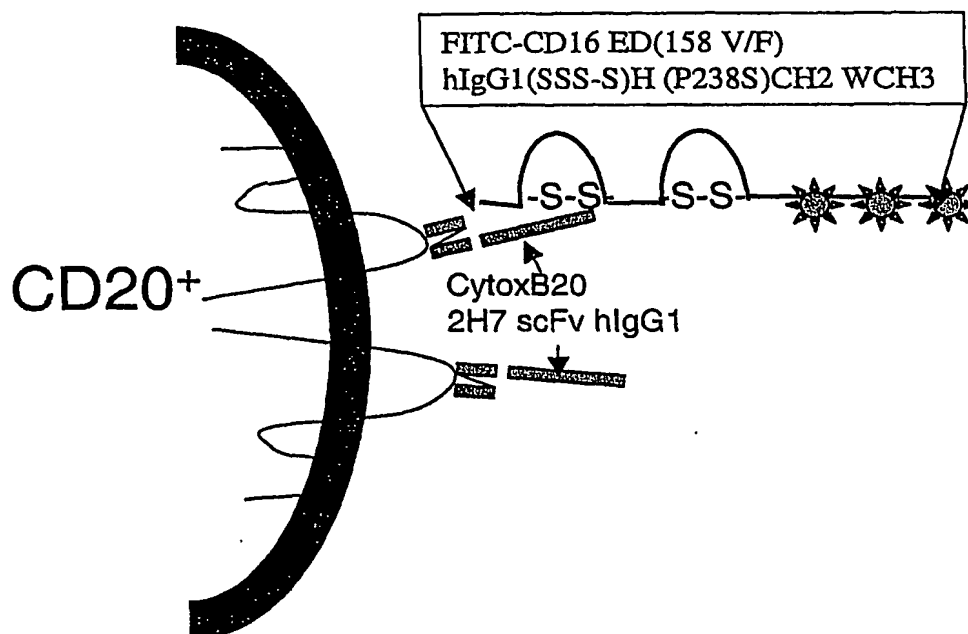


Fig. 70

Binding of FITC Labeled, Recombinant Human  
CD16(ED) extracellular domain -Ig Fusion Protein to  
CytoxB Derivatives on CD20 CHO Cells



Expression of surface displayed SMIPs links  
modified cDNAs with the altered fusion proteins

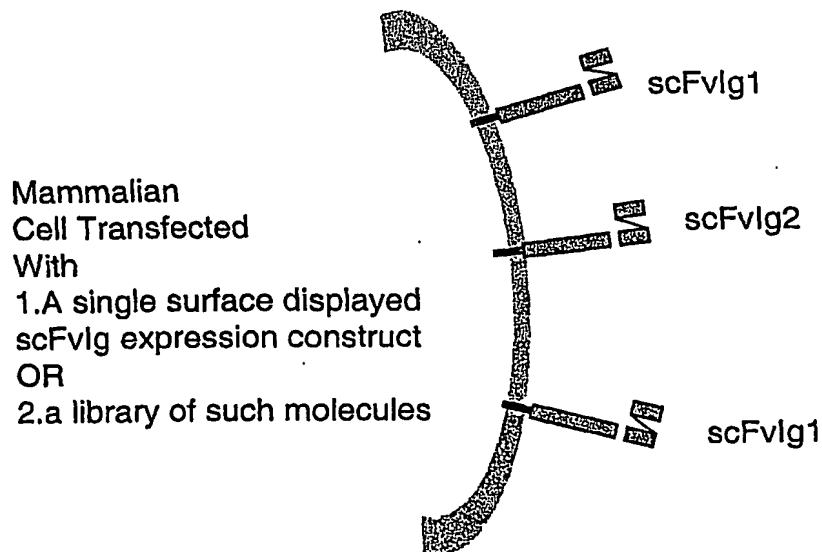


Fig. 71

## CD37 mAbs and scFvIg Induce Apoptosis

|        |                 |                    |                   |
|--------|-----------------|--------------------|-------------------|
| scFvIg | Bjab Staining   | Annexin V Positive |                   |
|        | No scFvIg       | 17.5               |                   |
|        | 2H7 MH          | 27                 |                   |
|        | G28-1 MH        | 30.6               |                   |
|        | G28-1 IgAH      | 28.9               |                   |
|        | HD37 MH         | 29.1               |                   |
|        | (2H7+G28-1)MH   | 41                 |                   |
|        | (2H7+HD37) MH   | 37.1               |                   |
|        | (G28-1+HD37) MH | 35.3               |                   |
|        |                 |                    |                   |
| mAbs   |                 |                    | plus GAM          |
|        | Ramos           | AnnexinV Positive  | AnnexinV positive |
|        | cells alone     | 3                  | 3.3               |
|        | 2H7 Mab         | 1.4                | 3.1               |
|        | G28-1 Mab       | 18.3               | 8.7               |
|        | HD37 Mab        | 3.7                | 3.1               |
|        | G28-5           | 3.9                | 8.3               |
|        | 2H7+G28-1       | 32.3               | 35.7              |
|        | 2H7+HD37        | 5                  | 10.5              |
|        | 2H7+G28-5       | 5.7                | 19.4              |
|        | HD37+G28-1      | 26.9               | 50                |
|        | HD37+G28-5      | 8.2                | 18.4              |
|        | G28-1+G28-5     | 39.5               | 68.3              |
|        |                 |                    |                   |
|        |                 |                    |                   |

Caspase 3 Activity in Ramos Cells after 4 Hour  
Incubation With CytoxB20G SMIP

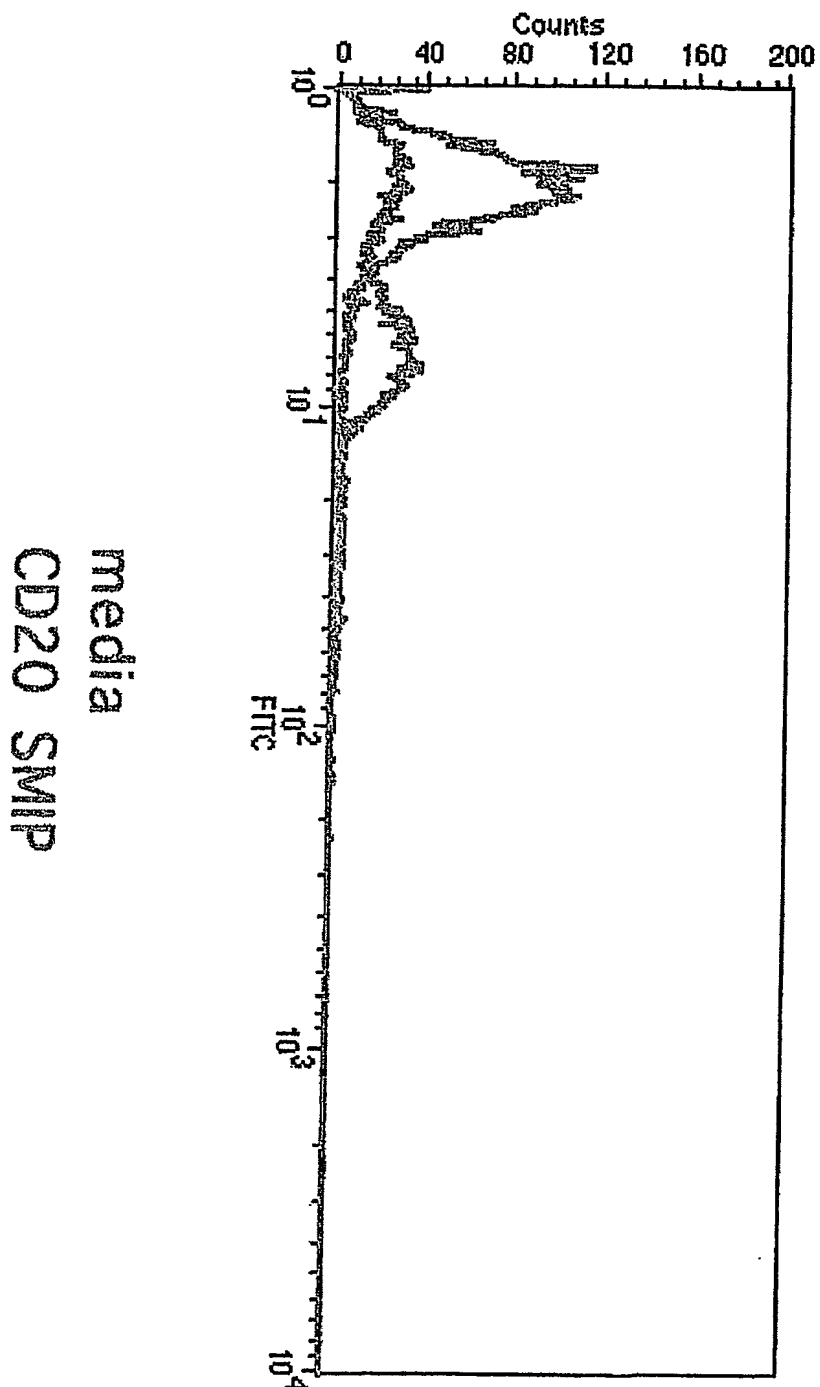


Fig. 72

# Complement Dependent Cytotoxicity Mediated by CytosB20G Derivatives

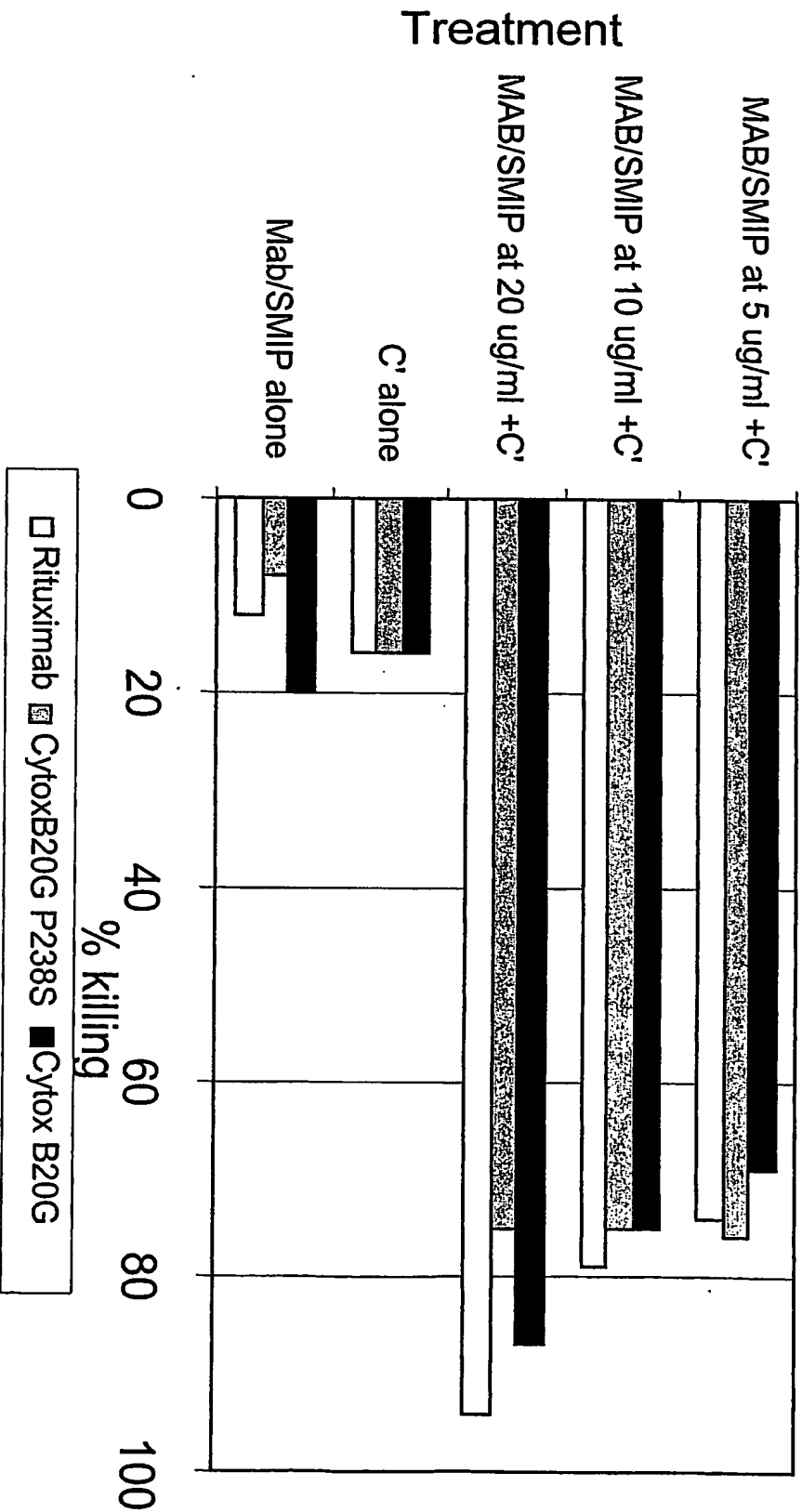


Fig. 73

Figure 76: CDC Activity of CytosB20G SMIPs. CytosB20G, CytosB20GP238, or Rituximab were incubated at increasing concentrations with 10<sup>4</sup> Bjab Target Cells and a 1:10 dilution of rabbit complement (PelFreez) in a volume of 100 microliters for sixty minutes. Aliquots were stained with trypan blue (Invitrogen), and counted using a hemacytometer to determine the percentage of the cell population killed during treatment. Negative controls with cells and only one reagent were also included.

# ADCC Activity of CytoxB20G SMIPs

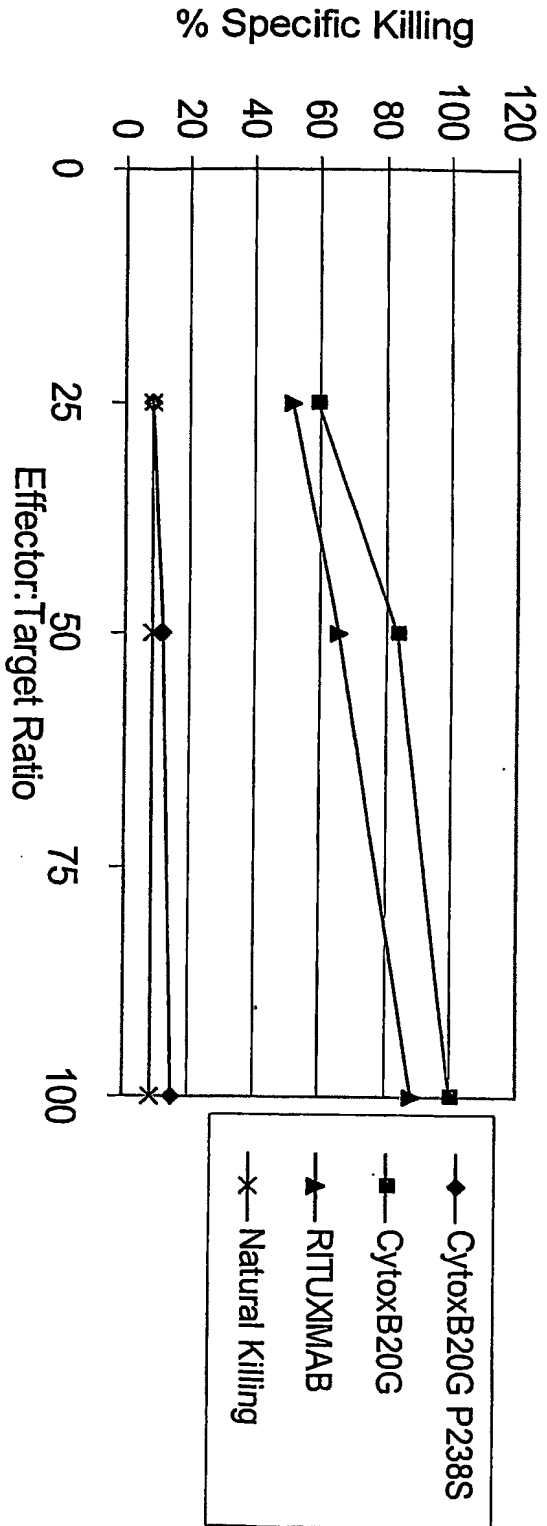


Fig. 74

Figure 77: ADCC Activity of CytoxB20G SMIPs. ADCC activity of CytoxB20G or Rituximab was measured *in vitro* against BJAB B lymphoma cell line as target and using fresh human PBMC as effector cells. Effector to target ratios were varied as follows: 100:1, 50:1, and 25:1, with the number of BJAB cells per well remaining constant but varying the number of PBMC. Bjab cells were labeled for 2 hours with  $^{51}\text{Cr}$  and aliquoted at a cell density of  $5 \times 10^4$  cells/well to each well of flat-bottom 96 well plates. Purified fusion proteins or rituximab were added at a concentration of 10  $\mu\text{g}/\text{ml}$ , and PBMC were added at 1.25  $\times 10^6$  cells/well (25:1), 2.5  $\times 10^6$  cells/well (50:1), or 5  $\times 10^6$  cells/well (100:1), in a final volume of 200  $\mu\text{l}$ . Natural Killing was measured at each effector:target ratio by omission of SMIP or Mab. Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells. Reactions were incubated for 5 hours, and 100  $\mu\text{l}$  culture supernatant harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released on a Packard Top Count NXT Microplate Scintillation Counter.

# Binding of soluble FITC-CD16 to Cytox B20G on CD20 CHO Cells

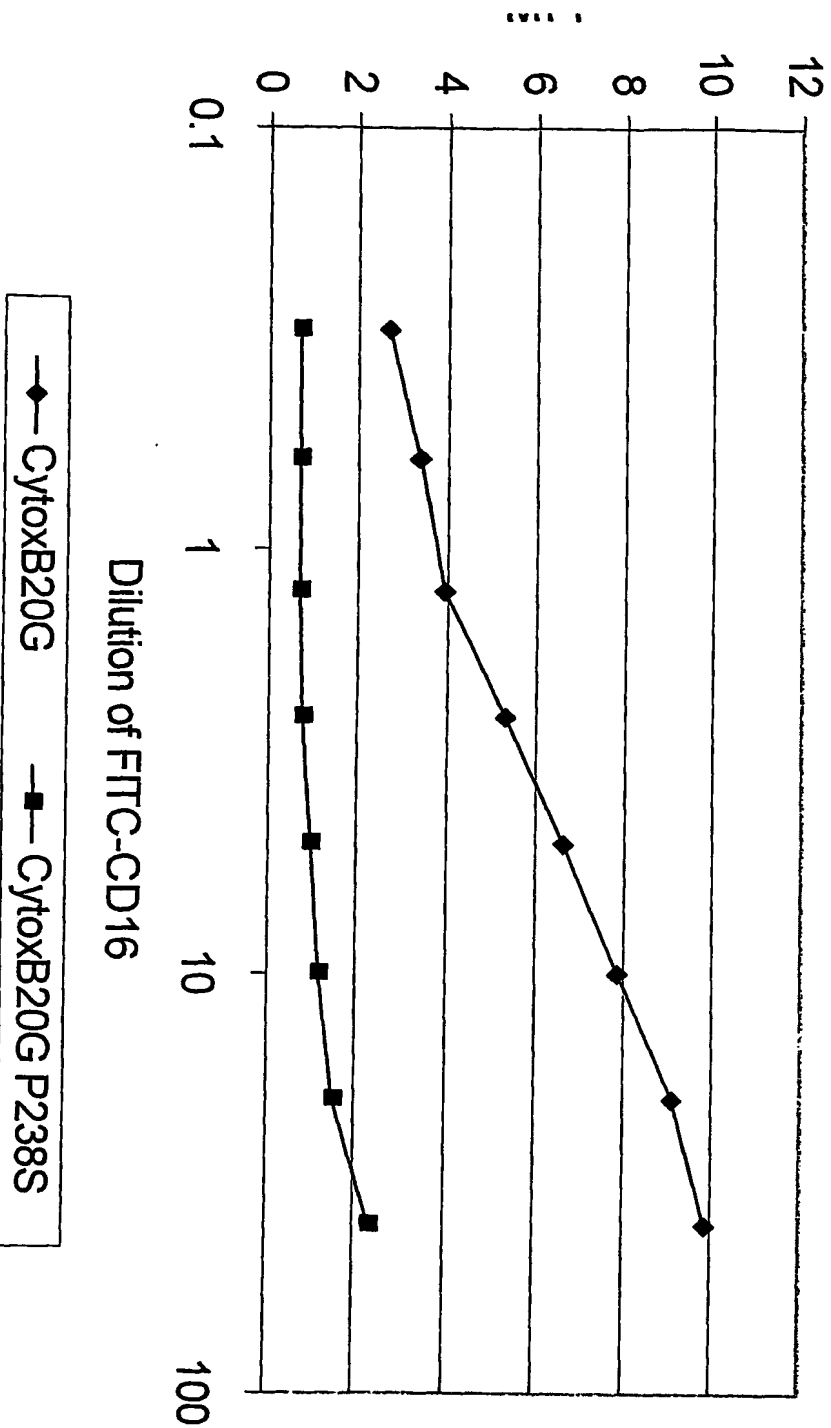


Fig. 75

re 78: Binding of soluble FITC-CD16 to Cytox B20G on CD20 CHO cells. CD20 CHO cells ( $10^6$ ) were incubated with saturating amounts of Cytox B20G or Cytox B20G P238S (10  $\mu$ g/ml) for one hour on ice in PBS/2% FBS. Cells were washed in PBS/2% FBS and incubated with serial dilutions of 0.5 mg/ml FITC-CD16 for one hour on ice. Cells were washed and specific binding measured by flow cytometry using a Beckman-Coulter Epics C machine. Results were analyzed using Expo analysis software and normalized fluorescence units graphed as a function of concentration.

# CytoxB20G and CytoxP238S bind to U937 Cells Expressing FcγRI High Affinity FcR

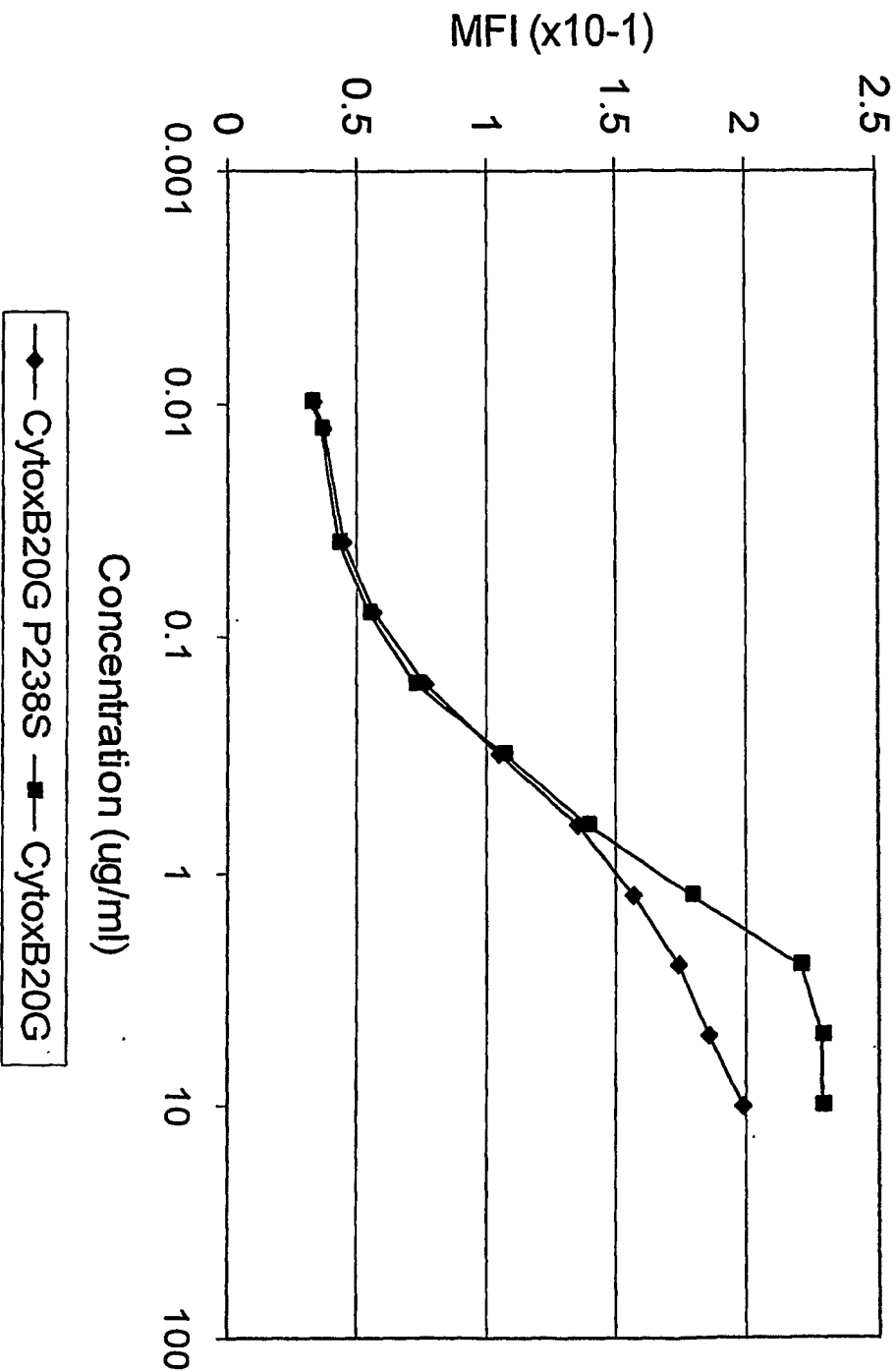


Fig. 76

re 79: CytoxB20G SMIPs bind similarly to U937 cells expressing the high affinity FcR (FcγRI, CD64). U937 cells expressing CD64 were incubated in PBS/2%FBS for one hour on ice with CytoxB20G orCytoxB20G P238S. Cells washed and incubated for one hour on ice with FITC-goat anti-human IgG1 (Fc specific) (Caltag ) at a final dilution 100. Cells were washed and fluorescence analysed on a Beckman-Coulter EpicsC flow cytometer. Data was analyzed using Expo analysis software, and fluorescence intensity graphed as a function of SMIP concentration.



# B Cell Depletion Mediated by Cytotoxic B20G SMIPs

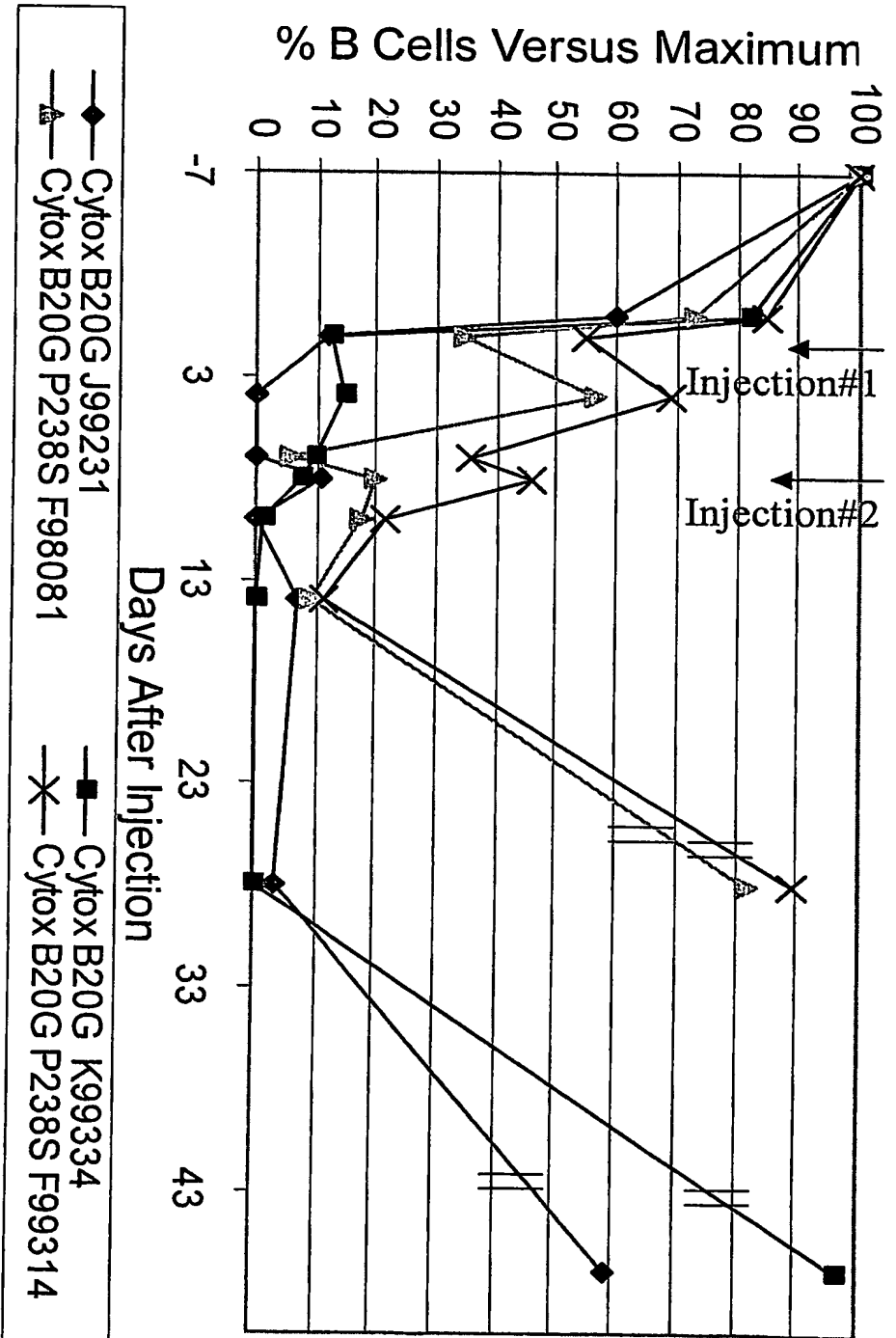


Fig. 77

10: Cytotoxic B20G or Cytotoxic B20G P238S were administered to macaques by intravenous injection at 6 mg/kg, with injections given one week apart. The effect on circulating B cells was measured by detection of CD40 positive B cells in peripheral blood. Blood samples were drawn from injected animals at days -7,0,1,3,7,8,10,14, 28, and 43. B cell number was estimated by performing CBC (complete blood counts) and two color flow cytometry analysis on monkey blood. Data are plotted as the number of CD40 positive blood B cells tabulated in thousands of cells per ml over time relative to the initial pre-injection time point level of B cells (maximum).

Figure 81: SEC on CytoxB37G SMIPs containing SSS and SSC hinge Domains from Human IgG1

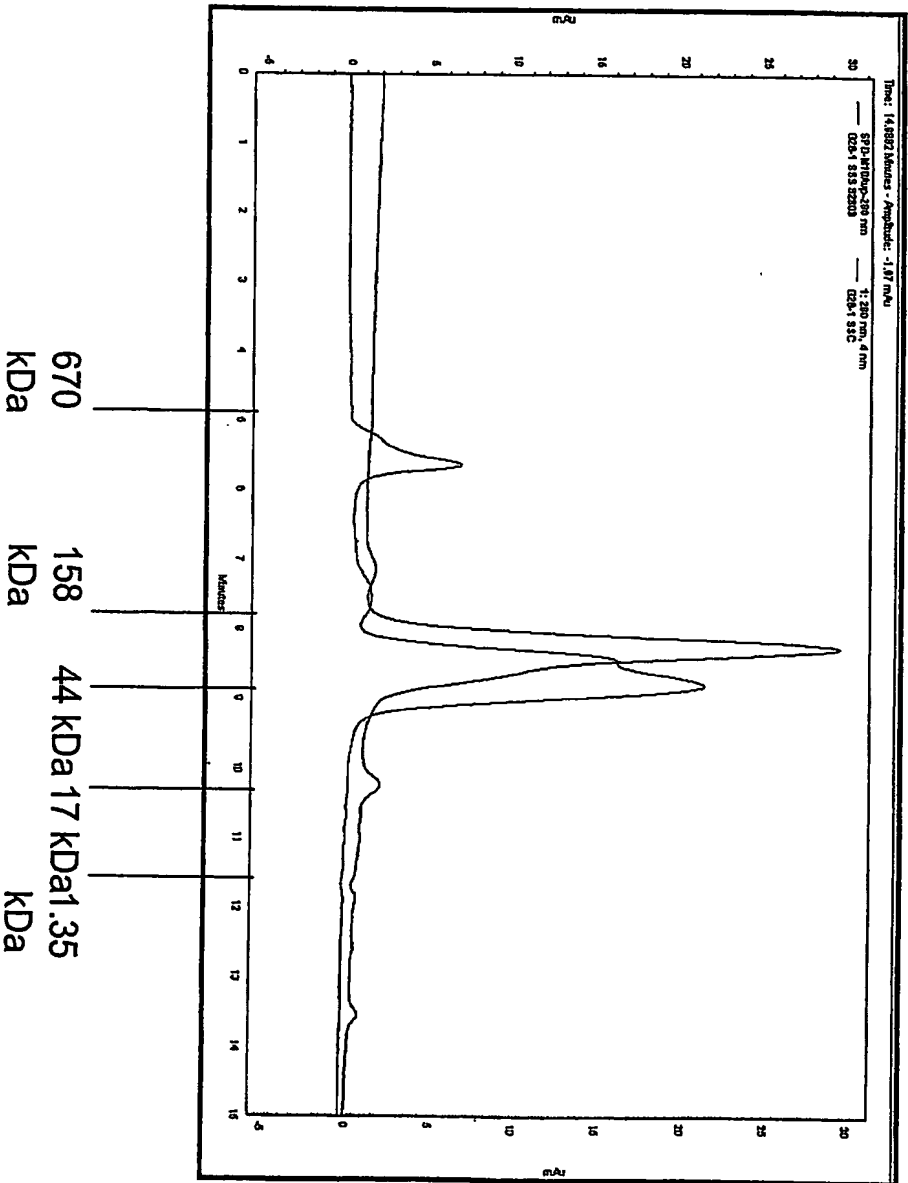


Fig. 78

Figure 81: SEC (Size Exclusion Chromatography) CytoxB37G SMIPs were purified from CHO culture supernatants by Protein A affinity chromatography. Purified aliquots of 10-25 µg were subjected to HPLC over a Tosoh Biosep, Inc. TSK 3000 SWXL HPLC column, pore size 5 µm. The flow rate was 1 ml/min, in PBS, pH 7.2 running buffer. Migration rates of molecular weight standards are indicated below the tracing. The CytoxB37G (SSS)H SMIP indicated in blue, while the CytoxB37G (CSS)H is indicated in red.

Figure 82: Binding of CytoxB37G SMIPs to B Cell Lymphoma Cell Lines

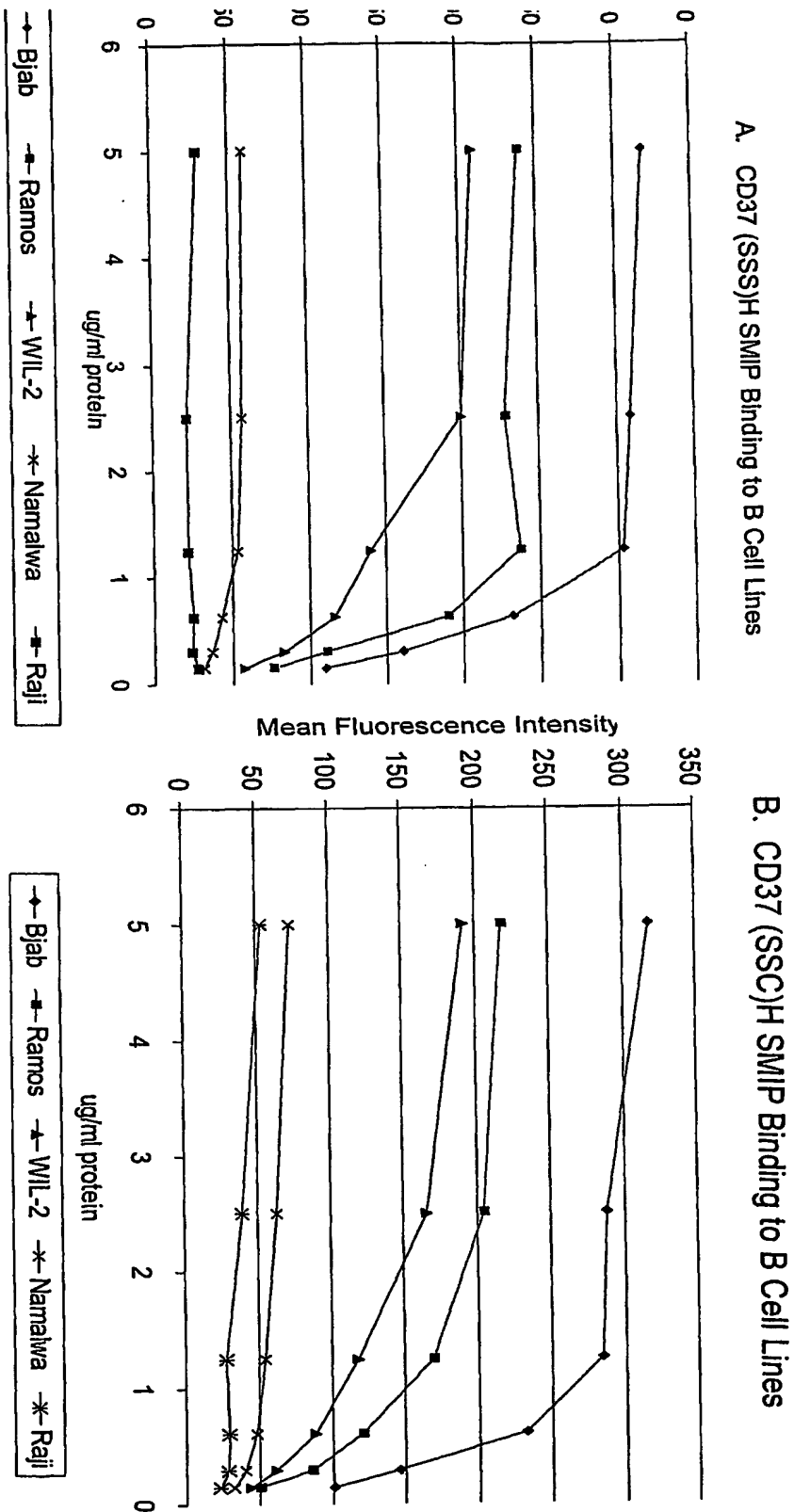


Fig. 79

ure 82: Binding of CytoxB37G SMIPs to B cell lymphoma cell lines. Serial dilutions of ified CytoxB37 (SSS)H G or CytoxB37 (SSC)H G SMIPs were incubated with  $10^6$  cells of each cell type for 60 minutes on ice in PBS/2%FBS. Samples were washed twice, and incubated with a mixture of FITC goat anti-human i and FITC goat anti-human IgG F(ab')<sub>2</sub> (CalTag) at 1:100 each, on ice for 45 minutes. Samples were washed and lyzed by flow cytometry using a FACsCalibur (Becton-Dickinson)

Fig. 80: AnnexinV-PI Staining of Ramos Cells Incubated  
24 hours with CD37 SMIPS

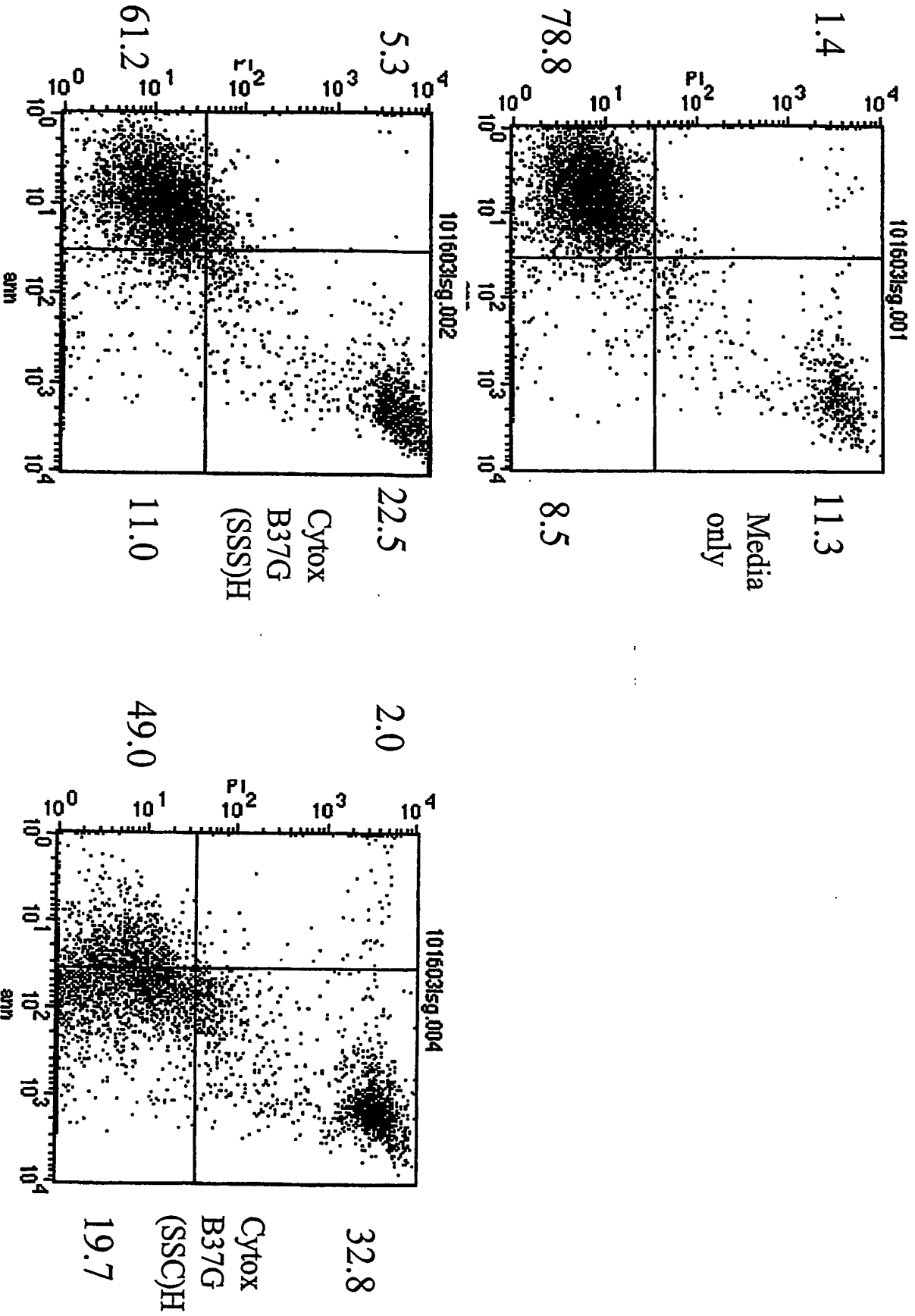


Figure 84: Thymidine Incorporation (Growth Inhibition) in Ramos B-cells after a 48 Hour Incubation with anti-CD37 SMIPs

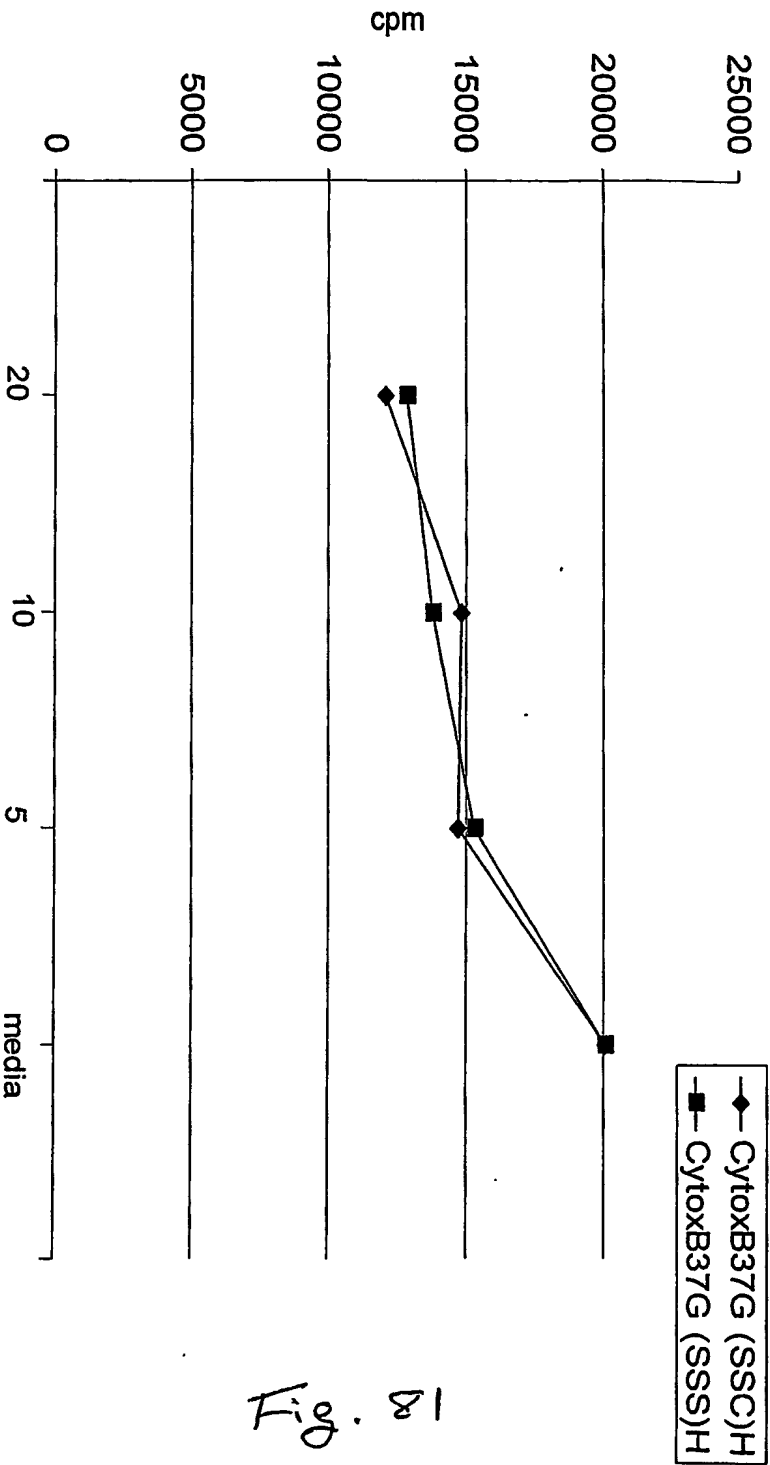


Figure 84: Ramos B cells were incubated with serial dilutions of purified CD37G SMIPs containing the IgG1 hinge identified as (SSS)H or (SSC)H. Cultures were incubated in 96 well flat bottom culture dishes (Costar) at 37°C, 5%CO<sub>2</sub> for 36 hours prior to pulsing with <sup>3</sup>H-thymidine for the 12 hours of a 48 hour incubation (0.75 µCi/well). Cells were harvested onto 96-well GFC plates using a Packard harvester, dried, and 25 µl Microscint scintillation fluid added to each well prior to counting on a TopCount NXT microplate (Packard) scintillation counter. Data are plotted as cpm incorporated versus protein concentration. Each SMIP show increasing inhibition of proliferation with increasing protein concentration.

Figure 85: The Induction of Apoptosis in Ramos B-cells after a 20 hour incubation with different combinations of CD20 and CD37 targeted SMIPs

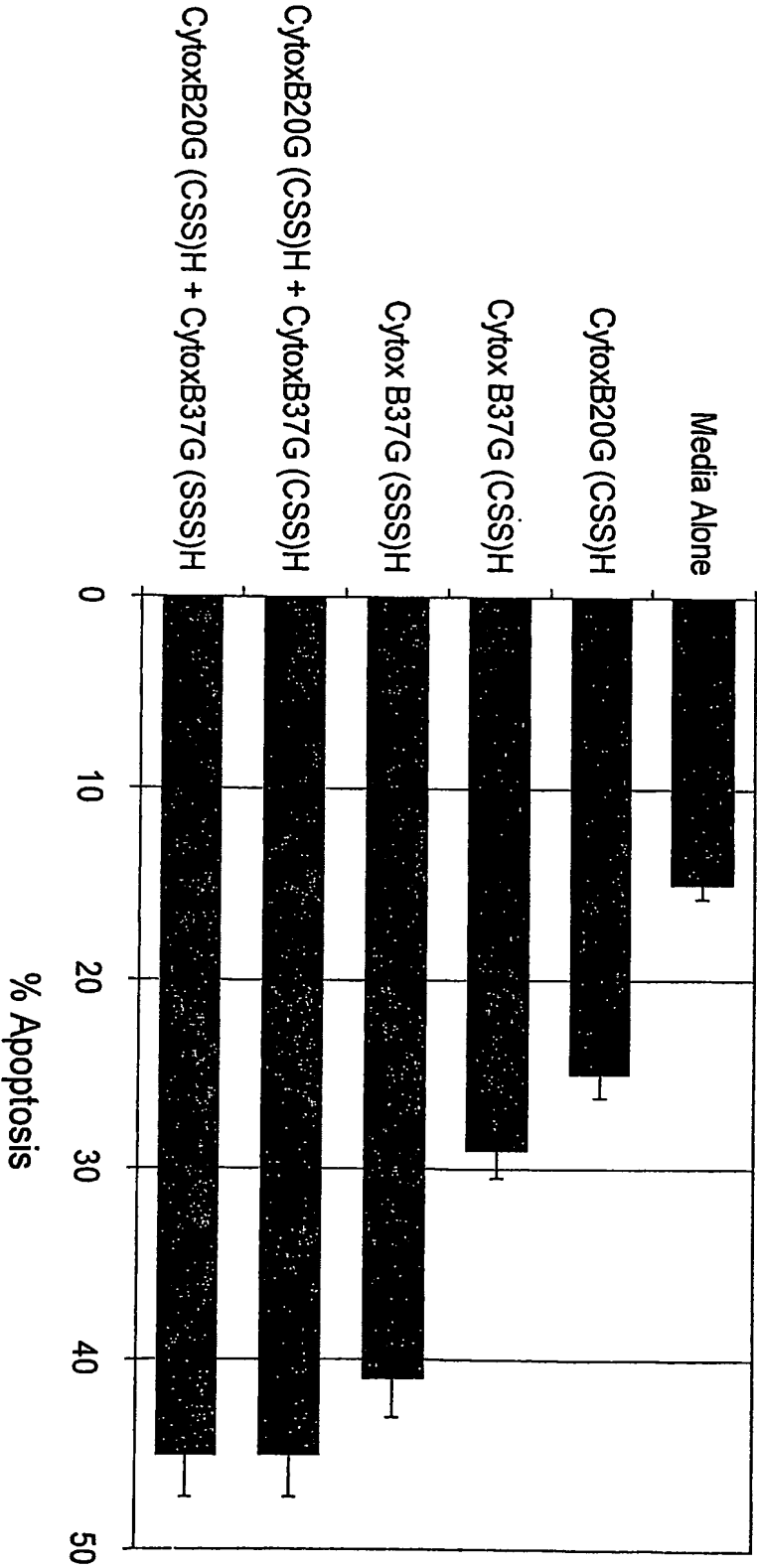


Fig. 82

Figure 85: Ramos B cells were incubated with CD20 and/or CD37 targeted SMIPs (10  $\mu$ g/ml) in solution for 20 hours. Cells were then harvested, washed, and incubated in annexin V and propidium iodide using a staining kit from Immunotech prior to two color flow cytometry using a FACScalibur flow cytometer (Becton-Dickinson). The graph shows the percentage of annexin V positive cells identified by their staining in the right quadrants of the dot plots.

Figure 86: Complement Mediated Killing of Ramos Cells by CD37 SMIPs

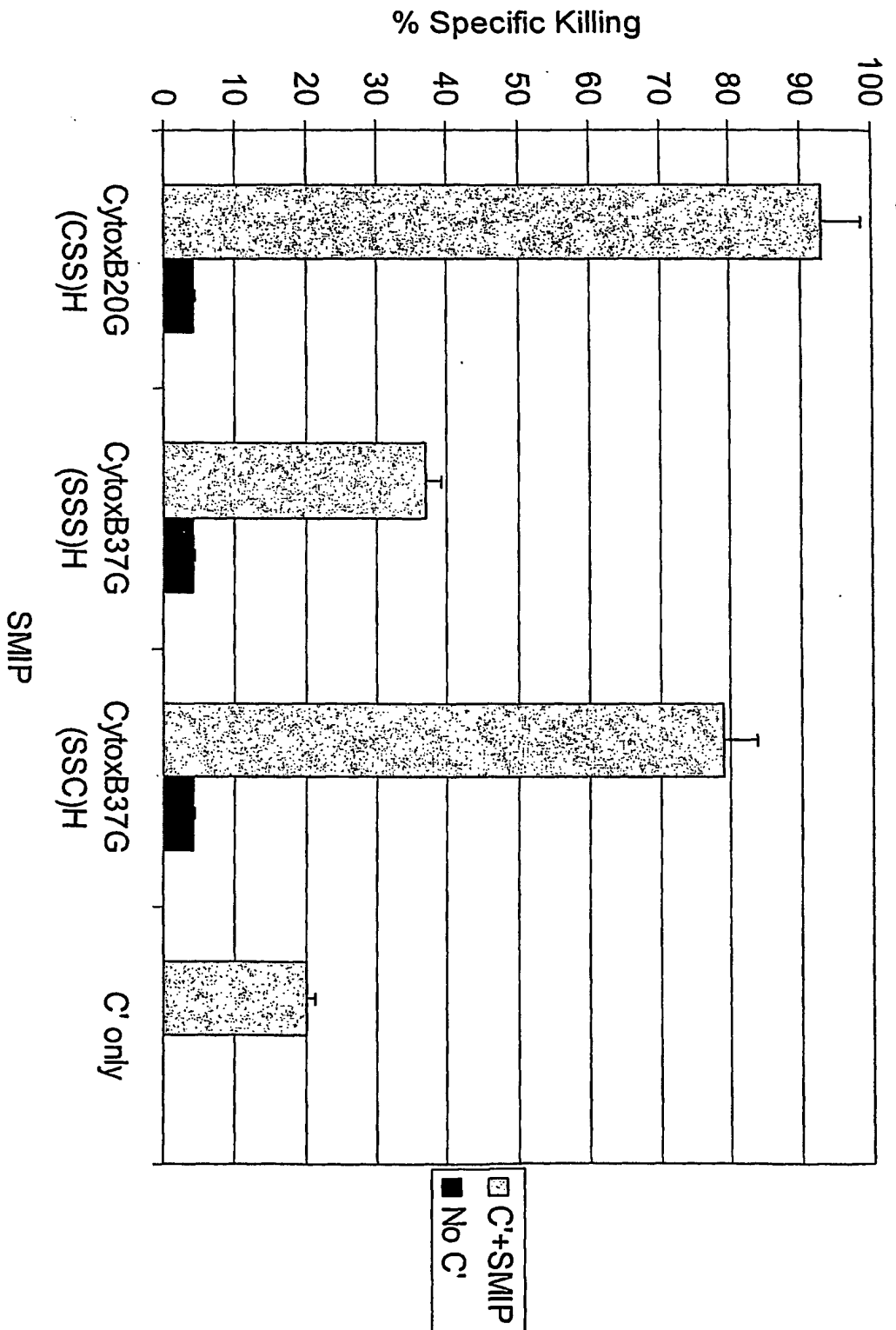


Fig. 83

Figure 86: CDC Activity of CytoxB37G SMIPs. CytoxB20G, CytoxB37 (SSS)H G, CytoxB37 (SCS)H, CytoxB37 (CSS)H, or CytoxB37 (SSC)H were incubated at 10  $\mu$ g/ml with 10<sup>4</sup> Ramos Target Cells and a 1:10 dilution of rabbit complement (PelFreez) in a volume of 150  $\mu$ l for 90 minutes. Aliquots were stained with trypan blue (Invitrogen), and counted using a hemacytometer to determine the percentage of the cell population killed during treatment. Negative controls with cells and only one reagent were also included.

Figure 87: ADCC Activity of CD37 SMIPs Against Ramos Targets

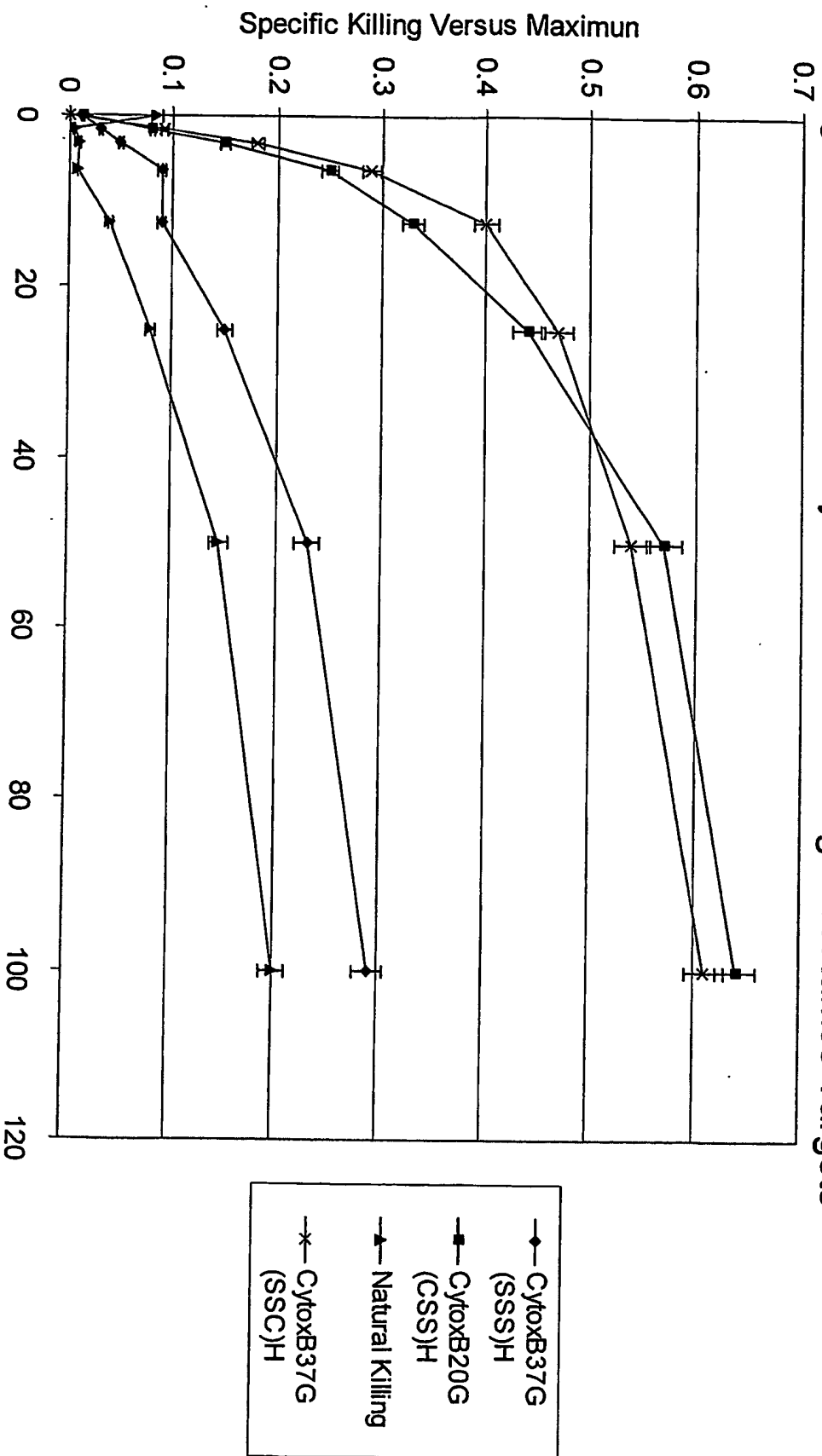


Fig. 84

Figure 87: CD37 SMIPs at 10  $\mu$ g/ml were incubated in flat-bottom 96 well plates with  $10^4$   $^{51}$ Cr-labeled Ramos cells and resting human PBMCs at different effector:target ratios ranging from 0 to 100. All incubations were performed in triplicate at each effector:target ratio. Natural Killing was measured at each effector:target ratio by omission of SMIP. Spontaneous release was measured without addition of PBMC or fusion protein, and maximal release was measured by the addition of detergent (1% NP-40) to the appropriate wells. Reactions were incubated for 6 hours, and 100  $\mu$ l culture supernatant harvested to a Lumaplate (Packard Instruments) and allowed to dry overnight prior to counting cpm released on a Packard Top Count NXT Microplate Scintillation Counter.



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